

EFFECT OF CIRCOVIRUS VACCINATION ON IMMUNE RESPONSES, VIRAL LOAD,
AND GROWTH PERFORMANCE OF PIGS UNDER FIELD CONDITIONS

by

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B.S., Purdue University, 2003
D.V.M., Purdue University, 2007

AN ABSTRACT OF A DISSERTATION

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Department of Diagnostic Medicine/Pathobiology
College of Veterinary Medicine

KANSAS STATE UNIVERSITY
Manhattan, Kansas

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Abstract

Vaccination against porcine circovirus type 2 (PCV2) has become a standard practice to improve pig mortality and growth rate in PCV2-affected herds. Unfortunately, there has been little field-based research evaluating factors which affect circovirus vaccination. The focus of this research was on potential vaccination-affecting factors such as age, dosing strategy, pig genetic makeup, and interaction with other vaccines. A total of 6,275 pigs were used to determine factors which affect circovirus vaccination and the effects of vaccination on average daily gain (ADG), immune responses, and viral circulation under field conditions. In the first study evaluating circovirus vaccination effects on PCV2 antibody titer, regardless of age and dose administration protocol, pigs vaccinated with a 2-dose circovirus vaccine had increased ($P \leq 0.008$) antibody titers compared with non-vaccinates. In a second study, dosing strategy failed ($P = 0.31$) to affect antibody titers. However, product and time after vaccination did affect ($P = 0.005$) antibody titers. In another 130-d study across the nursery and finishing phases, pigs vaccinated with a 2-dose circovirus vaccine had decreased ($P < 0.001$) serum PCV2 viral load compared with non-vaccinates and ADG of vaccinates was better than non-vaccinates. However, the effect was more pronounced (vaccination-by-genetic interaction, $P \leq 0.05$) in Duroc-based compared to Pietrain-based pigs. In a study limited to the nursery phase, vaccination for PCV2 and *Mycoplasma hyopneumoniae* independently reduced ADG and consumption, but the effect was product-dependent. In a 155-d study across the nursery and finishing phases, vaccination with a 2-dose, 2-vaccine program for PCV2 and *Mycoplasma hyopneumoniae* decreased ($P < 0.001$) nursery ADG but tended to increase ($P = 0.06$) finishing ADG compared to a 1-dose, 2-vaccine program, with no difference ($P = 0.66$) observed between final pig weights. Finally, circovirus vaccination affected PCV2-circulation in high-health research herds but not in a commercial herd where PCV2 DNA was detected in the environment. These results indicate that finishing performance was improved by a 2-dose circovirus vaccine; however, nursery performance was negatively affected by the same product. Circovirus vaccination responses of growth, viral load, and antibody titer were affected by pig genetic makeup, product, and PCV2-exposure status.

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Dedication

This dissertation is dedicated to my family and to the swine producers with whom I have had or will have the opportunity to work.

CHAPTER 1 - A field evaluation of porcine circovirus type 2 antibody production in pigs following circovirus vaccination using different vaccination strategies

Summary

Objective(s): To determine the effects of vaccination age, product, and dosing strategy on porcine circovirus type 2 (PCV2) indirect fluorescent antibody (IFA) titer and to determine important sources of variation when using the PCV2 IFA assay.

Materials and methods: In experiment one, 125 pigs were assigned to 1 of 4 vaccinated treatments or a non-vaccinated treatment (25 pigs per treatment). Vaccinates received Circumvent PCV (Intervet/Schering-Plough Animal Health, Millsboro, DE) at 1 and 3 or 3 and 5 wk of age, using either 2 mL or 1 mL per dose.

In experiment two, 90 pigs (15 pigs per treatment) were vaccinated intramuscularly with different circovirus vaccines and dosing strategies. Vaccine treatment designation were: (1) BI: CircoFLEX; Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO, (2) FD: Suvaxyn PCV2; Fort Dodge Animal Health, Fort Dodge, IA, or (3) IN: Circumvent PCV. Dosing strategies were: (1) Full Dose: vaccinated at 3 wk (BI, FD, and IN) and 5 wk (IN only) of age with label dose (BI: 1 mL per dose, FD and IN: 2 mL per dose), or (2) Split Dose: vaccinated at 3 and 5 wk of age (BI: 0.5 mL per dose, FD and IN: 1 mL per dose).

In experiment three, pigs and their dams were bled at weaning to determine on-farm and within-laboratory sources of variation to consider when using a PCV2 IFA assay.

Results: Experiment one results indicate that vaccination treatment affected ($P < 0.001$) PCV2 IFA titer, but age at vaccination or dose did not affect ($P \geq 0.18$) IFA titer.

Experiment two results indicate that PCV2 IFA titer was affected ($P = 0.005$) by product and time but was not affected ($P = 0.31$) by dosing strategy.

Experiment three results indicate that gender and laboratory factors were important sources of variation for PCV2 IFA testing.

Implications: Compared with non-vaccinated pigs, circovirus vaccination increased pig IFA titer regardless of vaccination age or dose amount. Antibody response depended upon the vaccine used and sampling time. Within-day and between-day laboratory factors were sources of variation for PCV2 IFA testing and should be considered during experimental design.

Key words: antibody, circovirus, IFA, PCV2, swine, vaccine

Circovirus vaccines can effectively reduce mortality and improve growth performance in immunized pigs (Horlen et al., 2008; Jacela et al., 2011). In general, response to vaccination can be determined by an antibody increase following vaccination. However, detection of an antibody rise does not always indicate protective immunity. In contrast, a lack of antibody increase may not always indicate a lack of protective immunity. Understanding diagnostic assays and their limitations and coupling results with clinical observations is important for determining efficacy of vaccine products. Currently several diagnostic tests are performed to measure PCV2 antibody levels. These tests include enzyme-linked immunosorbent assays (ELISA) and indirect fluorescent antibody (IFA) assays. It has been reported that some level of maternal-derived, passively-acquired PCV2 antibody as measured by ELISA generally signified protection (Opriessnig et al., 2006) but not always (McKeown et al., 2005). Therefore, some production systems have vaccinated pigs earlier than label directions with the intent to immunize pigs before pigs were naturally exposed to PCV2 field virus. Also, some producers have adopted a half dose administration protocol to either reduce costs or to extend the vaccine supply across more pigs. There are limited data concerning response to vaccination with different dose administration and timing strategies as well as with different circovirus vaccine products. Thus, the objectives of these studies were to: (1) determine effects of vaccinating pigs at younger ages on PCV2 antibody response, (2) evaluate the effects of split dose, half dose, or full dose strategies on PCV2 IFA titers for different circovirus vaccines, and (3) determine important sources of variation to consider when designing studies using PCV2 IFA titer as a response criterion.

Materials and Methods

Procedures used in all 3 studies were approved by the Kansas State University Institutional Animal Care and Use Committee. Experiments were performed in 3 different multi-site production systems which had historically been PCV2-positive with evidence of clinical circoviral disease.

In experiment one, a total of 125 pigs were used in a 17-wk trial to evaluate the effects of a 2-dose circovirus vaccine on antibody production. Pigs were selected from 25 litters (5 pigs per litter) within a single farrowing group. Pigs were randomly assigned within litters to 1 of 4 vaccination treatments or a non-vaccinated control treatment. Vaccinated treatments were arranged in a 2×2 factorial with factors of age at vaccination (Young or Old) and dose (Full or Half). A single circovirus vaccine was used. The circovirus vaccine was a killed, 2-dose, baculovirus-expressed, PCV2-capsid protein-derived vaccine (Circumvent PCV; Intervet/Schering-Plough, Millsboro, DE). Vaccine was administered to pigs as an intramuscular injection in the neck according to 1 of 2 age treatments. Age treatments were: (1) Young: vaccinated at 1 and 3 wk of age, or (2) Old: vaccinated at 3 and 5 wk of age. All pigs allotted to any vaccinated treatment received 2-doses of circovirus vaccine; however, the amount administered was altered according to the dose treatment. Pigs assigned to the Full treatment received 2 mL per dose while pigs assigned to the Half treatment received 1 mL per dose. Pigs were bled at 3 (weaning), 9 (end-of-nursery), and 18 (mid-finishing) wk of age. Serum was tested for PCV2 antibodies using the Kansas State Veterinary Diagnostic Laboratory (KSVDL) PCV2 IFA assay.

In experiment two, a total of 90 pigs were used in a 20-wk trial to evaluate the effects of 3 different circovirus vaccines and 2 different dosing strategies on PCV2 antibody production. Immediately after weaning, pigs were moved into a nursery with 24 pens and 25 to 27 pigs per pen. From each of 6 pens, 15 pigs were randomly selected and assigned to 1 of 6 vaccine treatments in a balanced incomplete design so that pigs of different treatments were comingled in each pen. The remaining pigs in the weaning group which were not placed on test were vaccinated with Circumvent PCV according to standard farm procedures. Treatments were arranged in a 3×2 factorial with vaccine product and dosing strategy as the factors for evaluation. Vaccine treatment designations were: (1) BI: CircoFLEX; Boehringer Ingelheim

Vetmedica, Inc., St. Joseph, MO, (2) FD: Suvaxyn PCV2; Fort Dodge Animal Health, Fort Dodge, IA, and (3) IN: Circumvent PCV; Intervet/Schering-Plough, Millsboro, DE. At the time of the study, vaccines were labeled as either a 1-dose product (BI and FD) or a 2-dose product (IN). Both BI and IN vaccines were baculovirus-expressed, PCV2-capsid protein-derived vaccines while the FD vaccine was a chimeric vaccine derived from PCV1 and PCV2. All vaccines were administered as intramuscular injections in the neck; however, the volume administered varied with dosing strategy (Full Dose or Split Dose). Pigs assigned to the Full Dose treatment received each product according to label dose (BI: 1 mL administered at 3 wk of age, FD: 2 mL administered at 3 wk of age, and IN: 2 mL per dose administered at 3 and 5 wk of age). Pigs assigned to the Split Dose treatment had volumes split over 2 doses given at 3 and 5 wk of age (BI: 0.5 mL per dose, FD: 1 mL per dose, and IN: 1 mL per dose). The Split Dose treatment protocol resulted in the pigs assigned to the IN-Split Dose treatment receiving half of the total vaccine label volume while pigs in the FD-Split Dose and the BI-Split Dose treatments received the total 1-dose administration label volume but split into 2 doses. Pigs were bled at 3 (weaning), 5, 11, and 23 wk of age and serum samples were tested for PCV2 antibodies using the KSVDL PCV2 IFA assay.

In experiment three, a total of 312 pigs (17 to 24 d of age) were used to characterize passively-acquired, maternal-derived antibody levels in offspring from first parity dams. A total of 146 barrows and 166 gilts were included which represented 52 litters over 4 consecutive wk of farrowing (13 randomly selected litters per wk). If available, a total of 3 barrows and 3 gilts from each litter were randomly selected and bled at weaning to measure PCV2 antibody levels. If there were less than 3 barrows or gilts available from a litter, then additional barrows or gilts were selected to ensure 6 pigs from each litter were bled. There were 9 litters with less than 3 barrows and 3 litters with less than 3 gilts available. There was one litter that had an extra gilt sampled and tested while a different litter had 1 less gilt sample tested due to serum tube breakage during mail transport. Serum was collected from the dams at the same time their pigs were bled. Dams in this system were vaccinated at 5 and 8 wk of age with Circumvent PCV (2 mL per dose) and once with Circumvent PCV (2 mL dose) during gilt-development at 27 wk of age. In addition to characterizing PCV2 antibody levels in the weaned pigs, both on-farm and within laboratory factors were identified as sources of variation during PCV2 IFA testing. This was achieved by performing IFA testing of the 312 weanling pig serum samples using a

crossover-type study design across 6 days of laboratory testing with 52 samples (1 pig from each litter with a total of 24 or 25 barrow and 27 or 28 gilt samples) tested per day. This design allowed measurement of the effects of gender and weaning wk as well as laboratory conditions such as IFA day and sample replication. Replication indicated the order of serum loading (first dispense or second) for the initial dilution when using an electronic, programmable pipette and running duplicate tests. To load the pipette, a total of 20 μ l was drawn into the pipette tip and the first dispense of 10 μ l was considered replication 1 and the second dispense of 10 μ l, which emptied the tip, was considered replication 2.

Diagnostic testing:

Antibodies against PCV2 were detected using the KSVDL IFA assay. Diagnostic testing methods were accepted and validated according to the American Association of Veterinary Laboratory Diagnosticians' standard requirements necessary for diagnostic laboratory accreditation. Antigen for the IFA assay was obtained by infecting ST cells with a low passage PCV2b virus. The cells were fixed in 80% acetone after 3 days of incubation and plates were stored at -20°C until use. For each experiment, serum samples were held at -80°C until all samples were collected at which point all serologic testing was performed.

Serial 1:2 dilutions were performed for the assay beginning with a 1:20 serum to PBS dilution. Plates were incubated for 1 hour at 37°C and then washed 2 times with PBS. Bound swine antibody was detected with FITC-conjugated goat anti-swine IgG (H+L) (Jackson ImmunoResearch Laboratory, West Grove, PA), diluted 1:75 in PBS with added Evan's Blue Dye. Plates were again incubated for 1 hour at 37°C and then washed 2 times. A 50% glycerol solution with PBS was added to each of the 96 wells. All plates were viewed under a fluorescence microscope and titers were determined by a single trained technician. Titration endpoints were calculated as the reciprocal of the last serum dilution that gave a positive fluorescence result.

Statistical analysis:

Prior to analysis, all IFA titers were \log_2 transformed to approximate a normal distribution of titers. For the IFA analysis, the \log_2 of 10 was used when serum samples did not have PCV2 antibody detected at the most concentrated dilution (1:20). The \log_2 of 5,120 was used when samples were strongly positive at the least concentrated dilution (1:2,560). These methods allowed those low antibody level (< 1:20) or high antibody level (> 1:2,560) results to

be weighted differently than samples with normal-intensity fluorescence detected at dilutions of 1:20 and 1:2,560. \log_2 transformed antibody titers were transformed back to the original scale for presentation as geometric mean titers.

For experiment one, IFA data were analyzed by repeated measures analysis of variance using the MIXED procedure in SAS version 9.1.3 (SAS Institute Inc., Cary, NC) with pig as the experimental unit. The model included fixed effects of vaccination treatment, time of sampling, and their interaction and a random effect of litter. Differences among treatments were determined using least squares means ($P < 0.05$). Single degree of freedom contrast statements were used to determine the main effects of age and dose on IFA titer.

For experiment two, IFA data were analyzed by repeated measures analysis of variance using the MIXED procedure in SAS version 9.1.3 with pig as the experimental unit. The model included fixed effects of vaccine treatment, dosing strategy, time of sampling, and their interactions. Nursery pen was included as a random effect. Differences between treatments were determined using least squares means ($P < 0.05$).

For experiment three, factors were analyzed by analysis of variance using the GLIMMIX procedure in SAS version 9.1.3 in a $2 \times 4 \times 6 \times 2$ factorial arrangement with fixed factors of gender (barrow or gilt), weaning wk (1, 2, 3, or 4), IFA laboratory testing day (1, 2, 3, 4, 5, or 6), replication (replication 1: first pipette dispense or replication 2: second pipette dispense), and all interactions. Litter was included as a random effect in the statistical model and pig was the experimental unit for analysis. The F-tests reported by type 3 tests of fixed effects in SAS were used to determine significant sources of variation ($P < 0.05$) for weaned pig IFA titer.

Results

For experiment one, there was no interaction ($P = 0.31$; Figure 1.1) observed between vaccination treatment and time for PCV2 IFA titer. In addition, there was no effect ($P = 0.80$) of sampling time for PCV2 IFA titer. Vaccination treatment affected ($P < 0.001$; Figure 1.2) the mean PCV2 antibody titer with non-vaccinated control pigs having decreased ($P \leq 0.008$) mean PCV2 antibody levels compared with any vaccinated pig treatment. Also, there was no effect of age at vaccination ($P = 0.95$) or dose ($P = 0.18$) for overall PCV2 IFA titer.

For experiment two, there was no 3-way interaction ($P = 0.12$; Figure 1.3) observed with vaccine treatment, dosing strategy, and time for IFA titer. There were no 2-way interactions

between dosing strategy and time ($P = 0.46$) or vaccine treatment and dosing strategy ($P = 0.15$) observed for IFA titer. There was a 2-way interaction ($P = 0.005$; Figure 1.4) between vaccine treatment and time observed for IFA titer. This interaction was the result of pigs vaccinated with IN vaccine having increased ($P \leq 0.04$) antibody levels at 3 and 5 wk of age compared with pigs vaccinated with BI vaccine with antibody levels of pigs vaccinated with FD vaccine intermediate. At 11 and 23 wk of age, pigs vaccinated with IN vaccine had increased ($P \leq 0.004$) antibody levels compared with pigs vaccinated with either BI or FD vaccines. As measured by the KSVDL PCV2 IFA assay, pigs vaccinated with BI or FD products did not demonstrate any PCV2 antibody increase by 11 or 23 wk of age. Thus, the magnitude of antibody response at 11 and 23 wk of age was greater for pigs vaccinated with IN vaccine than for pigs vaccinated with either BI or FD vaccine. Vaccine dosing strategy did not affect ($P = 0.31$) PCV2 antibody levels.

For experiment three, the distribution of PCV2 IFA titers from the 52 first parity dams sampled at the time their pigs were weaned was determined (Figure 1.5). The titers of the dams ranged from 80 to greater than 2,560. Regression analysis of the weanling pig IFA titers with the sow IFA titers indicated that sow antibody titer could explain approximately 37% of the variation associated with the pig IFA titer data (data not shown). For the multivariable model of pig IFA titer, there were no 4-way or 3-way interactions ($P \geq 0.84$; Table 1.1) with gender, weaning wk, IFA laboratory testing day, or replication for IFA titer. There were 2-way interactions between gender and IFA day ($P = 0.02$) and IFA day and replication ($P = 0.03$; Figure 1.6) observed for IFA titer. There was a trend for a 2-way interaction between gender and wean wk ($P = 0.06$; Figure 1.7) for IFA titer. There were no other 2-way interactions ($P \geq 0.19$) observed for IFA titer. Though also included in higher order interactions, IFA day and replication were highly significant ($P < 0.001$) as individual effects. In addition, litter accounted for approximately 50% of the variability which was not explained by the fixed effects (gender, weaning wk, IFA laboratory testing day, replication, and their interactions) in the model for weaned pig IFA titer.

Discussion

Since introduction of commercial circovirus vaccines, veterinarians and producers have been developing vaccination protocols with the intent to maximize vaccine efficacy and optimize

return on investment. In spite of manufacturers' label recommendations, age at vaccination, dose volume, and dose administration strategy are not consistent between production systems.

Field reports have suggested that high levels of passively-acquired PCV2 antibody interfered with circovirus vaccination and led to a poorer response to vaccination. Despite these reports, results from several studies do not support these suggestions. Horlen et al. (2008) reported increases in antibody titers after vaccination of pigs which were seropositive at the time of first vaccination with a 2-dose circovirus vaccine (Circumvent PCV) (Horlen et al., 2008). Fachinger et al. (2008) reported that vaccinating pigs with a 1-dose circovirus vaccine (CircoFLEX) when passively-acquired antibody levels were greater than 1:1,000 compared with vaccinating pigs when passively-acquired antibody levels less than 1:1,000 resulted in similar average daily gains. When comparing vaccinated pigs from both antibody level treatments to non-vaccinated pigs in similar antibody categories, average daily gains were increased for vaccinated pigs compared with controls (Fachinger et al., 2008).

Using the same 1-dose circovirus vaccine (CircoFLEX), Kixmüller et al. (2008) reported that level of passively-acquired antibody at the time of vaccination (IFA titer categories: less than 1:100, 1:100 to 1:1,000, greater than 1:1,000) did not affect overall PCV2 viremia reduction associated with circovirus vaccination under field conditions. However, increased concentration of passively-acquired antibody did delay onset of infection and lower overall viral load levels (Kixmüller et al., 2008). Determining that maternal-derived antibody delayed onset of infection suggested these passively-acquired antibodies provided some protection. These findings also supported research conclusions from McKeown et al. (2005) who reported that protection was dependent upon maternal-derived antibody level as determined by ELISA testing. In their study, high antibody levels were generally protective while low levels were not protective against experimental PCV2 infection (McKeown et al., 2005).

While passively-acquired, maternally-derived PCV2 antibody has been determined to provide some protection against PCV2 infection, some field reports also suggest that maternal-derived antibody inhibits response to some vaccines. However, association with a reduction in vaccine efficacy has not been reliably demonstrated under experimental PCV2-challenge.

Opriessnig et al. (2010) reported that vaccination of dams with 2 doses (off-label usage; 1 mL per dose) of a 1-dose circovirus vaccine (CircoFLEX) followed by vaccination of pigs with the 1 dose of the same product (1 mL per dose) resulted in no difference after PCV2 challenge

for antibody or serum viral load levels in pigs vaccinated and born of vaccinated dams compared with those of pigs vaccinated and born of non-vaccinated dams. Similar results were obtained when they performed similar procedures using Circovac (Merial, Lyon, France), a 2-dose (2 mL per dose) circovirus vaccine labeled in some countries (not the United States) for use on breeding animals (Opriessnig et al., 2010).

In a different challenge study, Opriessnig et al. (2008) demonstrated that vaccination with a 1-dose circovirus vaccine (Suvaxyn PCV2) in the presence of passively-acquired PCV2 antibody (positive indicating ELISA S/P ratios were equal to or greater than 0.300) resulted in similar viral load and histopathologic lesion reduction compared with responses of pigs vaccinated without presence of passively-acquired antibody (negative indicating ELISA S/P ratios less than 0.300). In the same study, pigs with passively-acquired antibody present at the time of vaccination had reduced severity of microscopic lymphoid lesions and lower PCV2 viral load compared with non-vaccinated pigs also having the presence of passively-acquired PCV2 antibody. This resulted in the conclusion that vaccination was not affected by presence of passively-acquired, maternal-derived PCV2 antibody at the time of vaccination (Opriessnig et al., 2008).

In a different study, vaccination with a 2-dose circovirus vaccine (Circumvent PCV) led to improvements in growth performance and mortality compared with non-vaccinated control pigs (Jacela et al., 2011). Using a PCV2 ELISA assay, there was demonstrable PCV2 antibody present in both the control (S/P ratio = 0.485) and vaccinated (S/P ratio = 0.364) pig serum at the time of vaccination. This suggests that despite vaccinating pigs with maternal-derived PCV2 antibodies, improvements are still achieved with vaccination. They concluded vaccine was effective despite the presence of passively-acquired PCV2 antibodies (Jacela et al., 2011).

Results from experiment one indicate that age did not affect mean antibody titer suggesting that a similar antibody level was achieved by pigs vaccinated at 1 wk of age or at the label recommended timing of 3 wk of age. It was not known in this study what the passively-acquired antibody levels were in the pigs vaccinated at 1 wk of age, but it would be assumed that they were similar to or higher than those observed for pigs at 3 wk of age. Regardless, results of our study indicate that overall antibody response to vaccination did not differ between pigs of different ages at vaccination. The lack of an interaction between treatment and wk indicates that antibody responses did not differ over time for the different treatments. However, vaccination

treatment affected the mean antibody level (Figure 1.2) as pigs from any vaccinated treatment had an increased mean antibody level compared with that of the non-vaccinated control pigs under conditions where, based on the antibody response for the control treatment, there appeared to be little exposure to field-strain PCV2 virus.

Although for both experiments one and two there were patterns in PCV2 antibody response which were suggestive of differences due to dose of vaccine, there was no effect of dose or dosing strategy detected for overall IFA titer. In experiment two, there were no interactions observed between dosing strategy and age or dosing strategy and vaccine treatment for PCV2 IFA titer. Dosing strategy also did not affect the overall antibody titer. Thus, for experiment two, antibody response to circovirus vaccination did not vary with dosing strategy to the extent it depended on vaccine product and time of sampling. Pigs vaccinated with Circumvent PCV had a detectable rise in antibody level by 11 wk of age while pigs vaccinated with CircoFLEX and Suvaxyn PCV2 did not have a detectable rise in antibody by any of the 3 post-vaccination sampling times (5, 11, or 23 wk of age; Figure 1.4). The severity of clinical circoviral disease experienced in this commercial herd, prior to the introduction of circovirus vaccines, deterred the use of a non-circovirus-vaccinated treatment as a control in our study. However, we believe the rise in antibody titer observed for the Circumvent PCV treatment was due to vaccination and not a result of natural PCV2 infection. If natural PCV2 exposure had occurred, a rise in antibody titer following natural viral exposure would have been expected in the CircoFLEX and Suvaxyn PCV2-vaccinated pigs because of an anamnestic response. In addition, there was no evidence of clinical circoviral disease observed during our trial. Thus, under conditions where there appeared to be little challenge from field strain PCV2 virus, antibody responses depended upon the vaccine product used but not necessarily the dosing strategy.

Regardless of the product used in our study, decay in antibody levels was apparent by 23 wk of age. In general, gilts are bred once they weigh 136 kg and gestation is approximately 16.5 wk long. With PCV2 antibody levels and decay similar to those observed in our study, when the time to breeding and subsequent gestation is considered, the amount of vaccine-induced antibody that would be passively transferred to piglets would be low. Therefore, if increased levels of vaccine-induced antibody in colostrum are desired then it may be necessary to administer a booster vaccine to breeding age females.

Although by 23 wk, a decreased in antibody levels was evident in pigs regardless of the vaccine used, the antibody responses in the 11 wk following vaccination in our study differed from those reported by Opriessnig et al. (2009). Comparing Circumvent PCV (2 doses; 2 mL per dose), Suvaxyn PCV2 (1 dose of 2 mL), Suvaxyn PCV2 (2 doses; 1 mL per dose), and CircoFLEX (1 dose of 1 mL) in a PCV2-challenge model and testing for PCV2 antibodies using an ELISA assay, they reported significant differences in antibody response at 7.5 wk of age for 1-dose compared with 2-dose products. Pigs receiving 1-dose products at 3.5 wk of age had a reduced antibody response compared with pigs receiving 2-dose products administered at 3.5 and 6.5 wk of age. These differences disappeared by 12.5 wk of age (Opriessnig et al., 2009). However, prior to this time, vaccination with any circovirus vaccine product produced a rise in antibody levels. It is unknown why, under experimental conditions and testing for antibody using an ELISA test, the circovirus vaccines tested resulted in increased antibody production while in our study, under field conditions and testing for antibody using an IFA assay, pigs vaccinated with Suvaxyn PCV2 or CircoFLEX failed to have a detectable rise in antibody levels.

There are little published data comparing PCV2 antibody assays or describing sources of variation to consider when performing or analyzing these tests. There have been comparisons of PCV2 ELISA and polymerase chain reaction (PCR) tests performed to evaluate test-to-test or laboratory-to-laboratory differences (Harding et al., 2009; Patterson et al., 2008); however, limited research has evaluated within-assay sources of variation. Results from experiment three indicate that gender had a tendency to affect antibody levels at weaning and should be considered during the design of trials involving IFA testing. There was a strong litter effect for PCV2 IFA titer of pigs which should be considered when designing trials. Approximately 50% of the variability not explained by the fixed effects in the statistical model was explained by the litter effect. This strongly suggests that litter-to-litter effects should be considered when selecting animals for use in a PCV2 antibody study because it appeared that the within-litter variation tended to be less than or equal to the between-litter variation. Thus, selecting multiple pigs from a single litter and placing more of them on one treatment than another may reduce variation associated with the treatment with a higher percentage of littermates and lead to detection of differences which may not be true (Type 1 error). Therefore, it is important to represent litters equally among treatments to avoid drawing false conclusions. In contrast, if the effects of litter are not accounted for in the experimental design and analysis, the variation not

explained by the fixed or random effects could increase and result in the failure to detect true treatment differences (Type 2 error) and require increased sample sizes to detect significant treatment effects.

Results from our third experiment indicate that experimental control should not conclude at the field level. The farm level litter of origin, gender, and potentially wean wk were all important sources of variation to consider during experimental design; however, sources of variation (IFA testing day and replication) also extended into the diagnostic laboratory. Although not a testable factor in our study, changing technicians within a study could add another source of variation for IFA testing because of the level of subjectivity in determining level of fluorescence. Of other factors identified as potential sources of variability, replication and IFA day explained a significant amount of variability. In our study, IFA day likely represented several factors, one of which was batch of IFA plates. A different batch of plates was used on days 4 and 5 and it appeared the IFA test results obtained on those 2 days were lower than on other days (Figure 1.6). Therefore, we believe that performing testing on serial samples not only within a day but also within a batch of plates is important. If serial samples are tested on different days for a single animal then false increases or decreases in antibody titers may be observed depending on laboratory conditions and materials. In randomized trials with different treatments, it is important to distribute the number of samples from the treatments across the days of testing so every testing day has equal representation across treatments.

For our experiment, replication also explained a significant amount of variation. Although the source of this variation was not known, when performing tests in duplicate and in a manner similar to the procedures used in our study, comparing results within replication or dispense order is important.

Laboratory techniques which have potential to influence results must be identified because accuracy of diagnostic tests and research results depend upon these procedures. In a research trial, running serial samples for individual pigs on a single IFA plate and blocking by testing day would help to account for some of this explainable variation.

If it is not possible to control within-day and between-day laboratory effects for the IFA testing procedures, then these factors should be documented and included in the statistical model to determine whether they had a significant effect on the measured response. Identification of conditions under which the responses were different would be important to interpretation and

comparison of IFA testing results. Recognizing that IFA results vary based on many factors makes it critical for veterinary clinicians to become actively involved in the diagnostic testing process in order to understand the procedures which occur in the laboratory and to be aware of the limitations of a diagnostic test.

Results from our studies indicate that, regardless of age at administration or dose, there were differences in antibody responses to circovirus vaccination compared with non-vaccinated pigs. In addition, antibody responses as measured by IFA assay to vaccination were product-dependent. Furthermore, our results indicate that IFA testing day, replication, litter of origin, gender, and potentially weaning wk all affect pig IFA titers and are important factors to consider when designing an experiment with IFA titer as a response variable.

Implications

- Circovirus vaccine administration strategy increased geometric mean IFA titer in vaccinated pigs regardless of age at injection or dose amount compared with pigs not vaccinated.
- PCV2 antibody response depended on both circovirus vaccine and time of sampling.
- When performing IFA assays within the diagnostic laboratory, both within-day and between-day variation affected results of PCV2 IFA testing. When designing experiments, day of IFA testing, replication, litter of origin, gender, and wean wk were important and should be considered as sources of variation for IFA testing.

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Figure 1.1 Indirect fluorescent antibody (IFA) geometric mean titers (GMT) at 3, 9, and 18 wk of age for pigs of different vaccination treatments (Exp. 1).

A total of 125 pigs were randomly assigned within litter (5 pigs per litter) to 1 of 4 vaccination treatments or a non-vaccinated control. Vaccination treatments consisted of different vaccine doses and ages at administration. The doses of circovirus vaccine (Circumvent PCV; Intervet/Schering-Plough Animal Health, Millsboro, DE) were: (1) Full: 2 mL per dose administered twice, (2) Half: 1 mL per dose administered twice. The ages at vaccination were: (1) Young: 1 and 3 wk of age; or (2) Old: 3 and 5 wk of age. Serum was collected at 3, 9, and 18 wk of age and tested for antibody by porcine circovirus type 2 IFA assay. Log₂ transformed individual pig IFA data were analyzed by repeated measures analysis of variance using the MIXED procedure of SAS version 9.1.3 (SAS Institute Inc., Cary, NC). The model included fixed effects of treatment, time of sampling, and their interaction and a random effect of litter. Resulting means were transformed back to the original scale for presentation as GMT.

Treatment × Time: $P = 0.31$

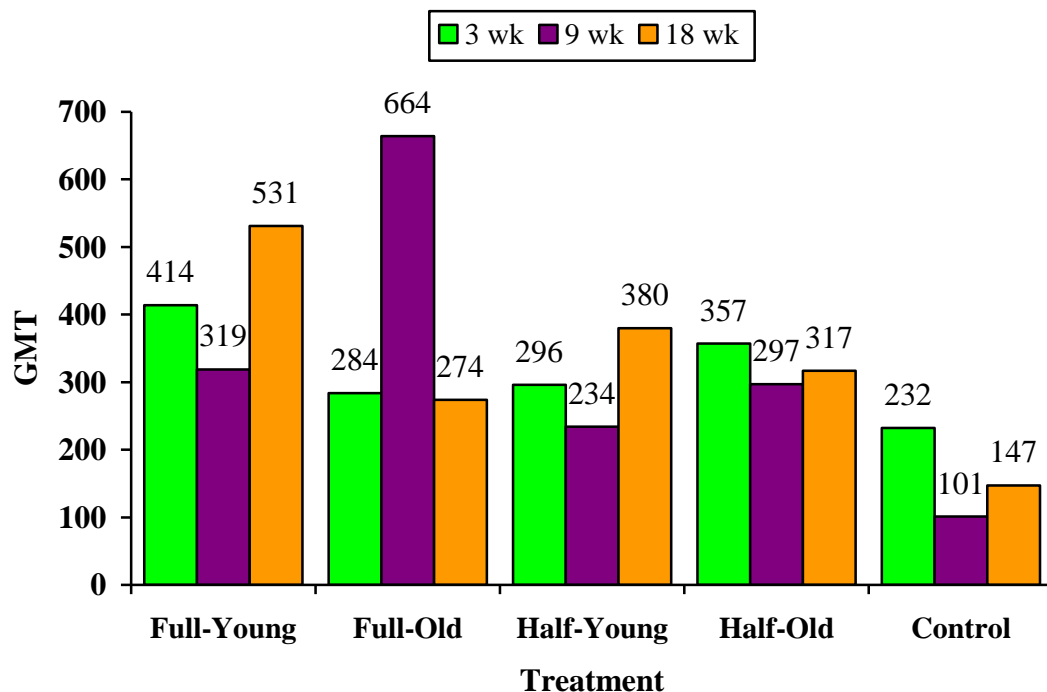


Figure 1.2 Average indirect fluorescent antibody (IFA) geometric mean titers (GMT) for pigs of different vaccination treatments (Exp. 1).

A total of 125 pigs were randomly assigned within litter (5 pigs per litter) to 1 of 4 vaccination treatments or a non-vaccinated control. Vaccination treatments consisted of different vaccine doses and ages at administration. The doses of circovirus vaccine (Circumvent PCV; Intervet/Schering-Plough Animal Health, Millsboro, DE) were: (1) Full: 2 mL per dose administered twice, (2) Half: 1 mL per dose administered twice. The ages at vaccination were: (1) Young: 1 and 3 wk of age; or (2) Old: 3 and 5 wk of age. Serum was collected at 3, 9, and 18 wk of age and tested for antibody by porcine circovirus type 2 IFA assay. Log₂ transformed individual pig IFA data were analyzed by repeated measures analysis of variance using the MIXED procedure of SAS version 9.1.3 (SAS Institute Inc., Cary, NC). The model included fixed effects of treatment, time of sampling, and their interaction and a random effect of litter. Resulting means were transformed back to the original scale for presentation as GMT.

Treatment: $P < 0.001$

^{a,b} $P < 0.05$

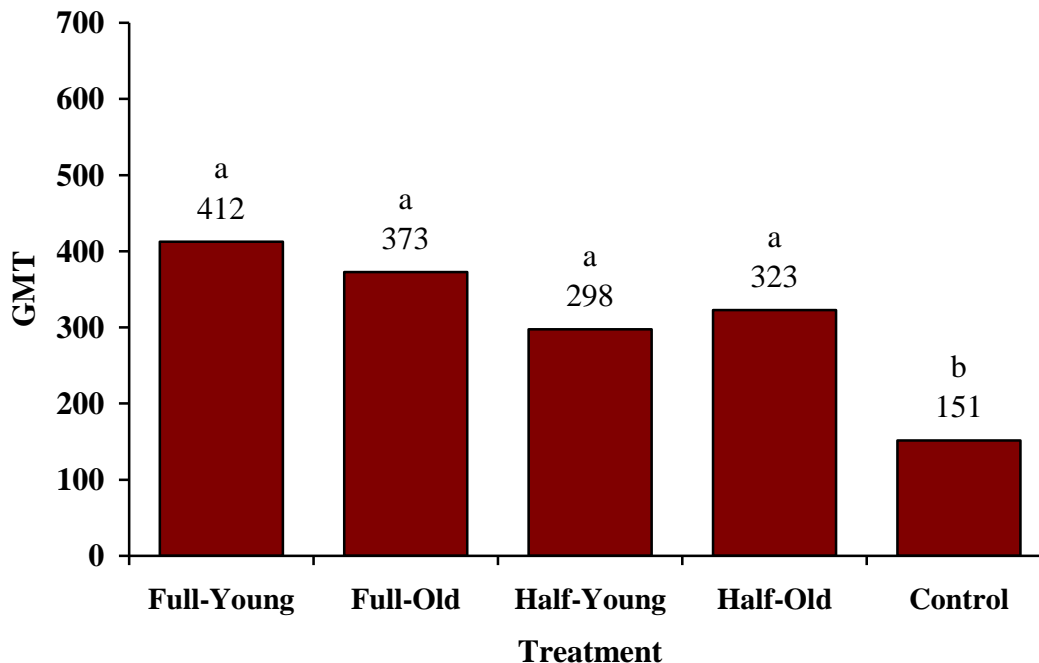
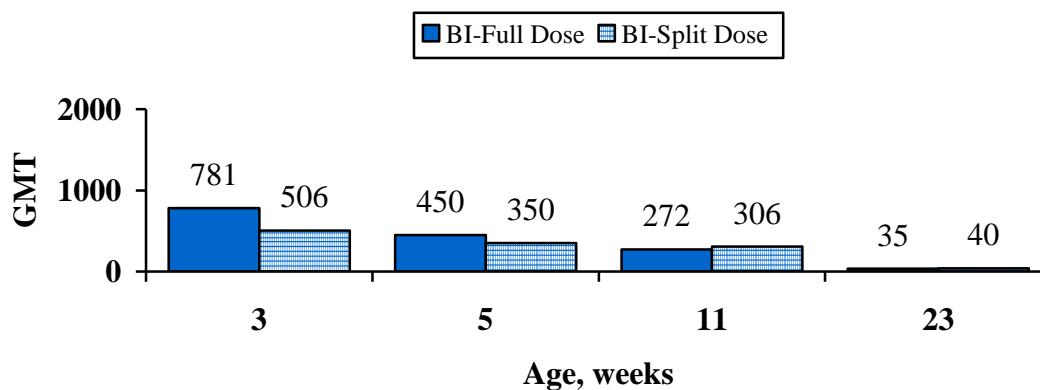


Figure 1.3 Indirect fluorescent antibody (IFA) geometric mean titers (GMT) at 3, 5, 11, and 23 wk of age for pigs administered different circovirus vaccines using different dosing strategies (Exp. 2).

A total of 90 pigs (15 randomly selected pigs in each of 6 pens) were assigned to 1 of 6 vaccination treatments with 3 pens containing 2 treatment replicates and 3 pens containing 3 replicates. Vaccination treatments consisted of different circovirus vaccines and dosing strategies. Vaccine treatment designations were: (1) BI: CircoFLEX; Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO, (2) FD: Suvaxyn PCV2; Fort Dodge Animal Health, Fort Dodge, IA, and (3) IN: Circumvent PCV; Intervet/Schering-Plough, Millsboro, DE. Dosing strategies were: (1) Full Dose (BI: 1 mL administered at 3 wk of age; FD: 2 mL administered at 3 wk of age; and IN: 2 mL per dose administered at 3 and 5 wk of age), or (2) Split Dose (BI: 0.5 mL per dose administered at 3 and 5 wk of age, FD: 1 mL per dose administered at 3 and 5 wk of age, and IN: 1 mL per dose administered at 3 and 5 wk of age). Pigs were bled at 3, 5, 11, and 23 wk of age and antibody levels were determined in serum samples using a porcine circovirus type 2 IFA assay. Individual pig IFA data were \log_2 transformed and then were analyzed by repeated measures analysis of variance using the MIXED procedure in SAS version 9.1.3 (SAS Institute Inc., Cary, NC) with pig as the experimental unit. The model included fixed effects of vaccination treatment, dosing strategy, time of sampling, and their interactions. Nursery pen was included as a random effect. Resulting means were transformed back to the original scale for presentation as GMT.

Treatment \times Dosing strategy \times Time: $P = 0.12$



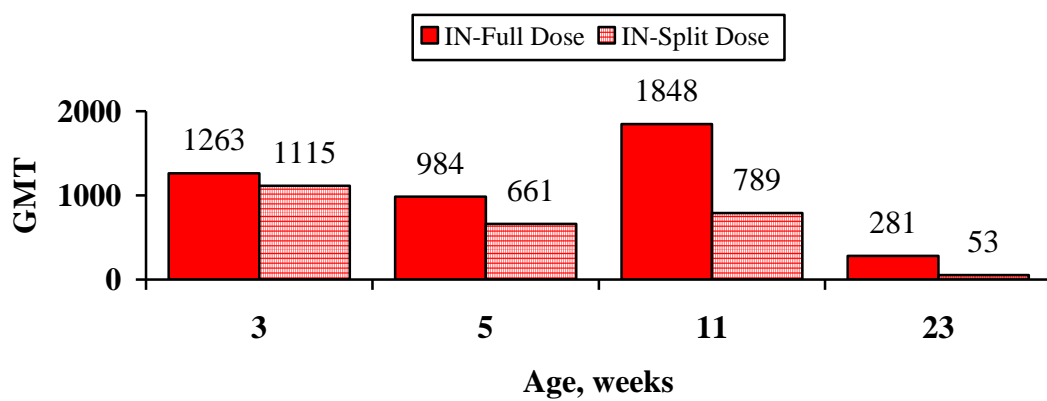
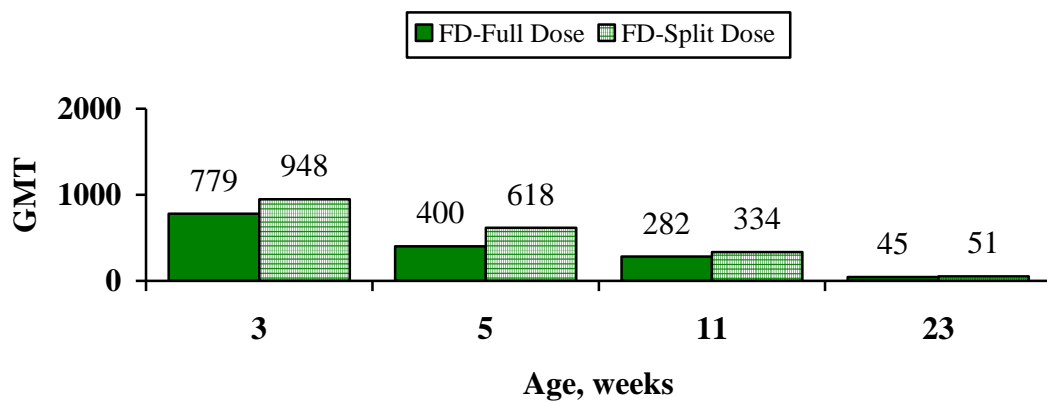


Figure 1.4 Indirect fluorescent antibody (IFA) geometric mean titers (GMT) at 3, 5, 11, and 23 wk of age for pigs administered different circovirus vaccines (Exp. 2).

A total of 90 pigs (15 randomly selected pigs in each of 6 pens) were assigned to 1 of 6 vaccination treatments with 3 pens containing 2 treatment replicates and 3 pens containing 3 replicates. Vaccination treatments consisted of different circovirus vaccines and dosing strategies (Full or Split Dose). Vaccine treatment designations were: (1) BI: CircoFLEX; Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO, (2) FD: Suvaxyn PCV2; Fort Dodge Animal Health, Fort Dodge, IA, and (3) IN: Circumvent PCV; Intervet/Schering-Plough, Millsboro, DE. Pigs were bled at 3, 5, 11, and 23 wk of age and antibody levels were determined in serum samples using a porcine circovirus type 2 IFA assay. Individual pig IFA data were log₂ transformed and then were analyzed by repeated measures analysis of variance using the MIXED procedure in SAS version 9.1.3 (SAS Institute Inc., Cary, NC) with pig as the experimental unit. The model included fixed effects of vaccination treatment, dosing strategy, time of sampling, and their interactions. Nursery pen was included as a random effect. Resulting means were transformed back to the original scale for presentation as GMT.

Treatment × Time: $P = 0.005$

a,b,c,d,e,f,g,h $P < 0.05$

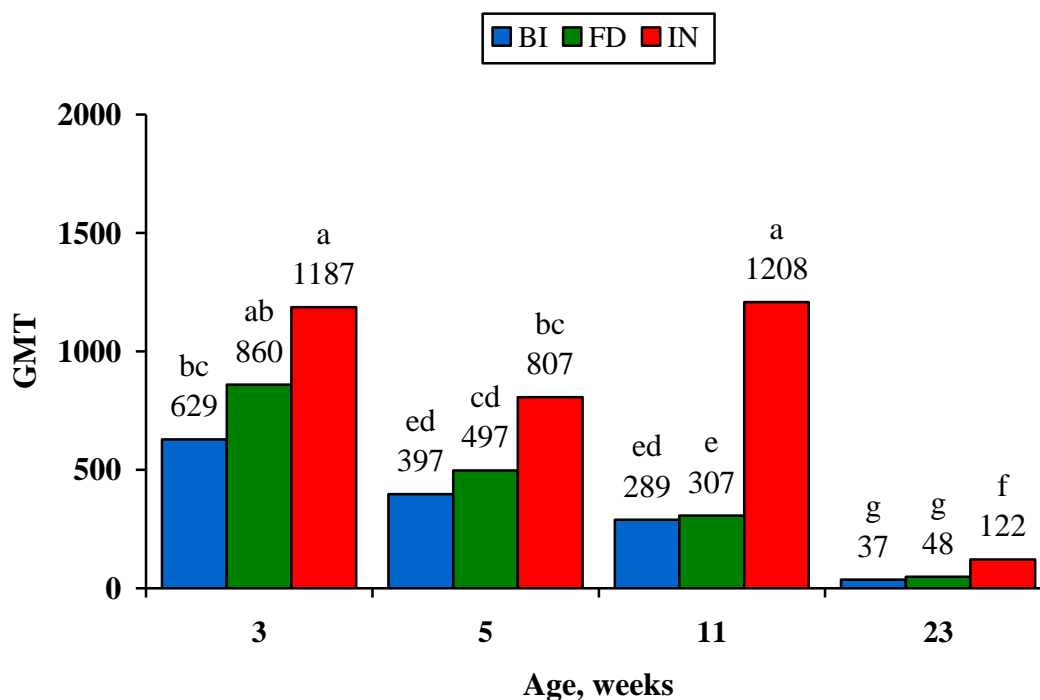


Figure 1.5 Distribution of porcine circovirus type 2 (PCV2) indirect fluorescent antibody (IFA) titers from circovirus-vaccinated first parity sows (Exp. 3).

A total of 52 first parity sows (13 sows per wk over 4 consecutive wk) were randomly selected at the time of weaning and had serum collected and tested by PCV2 IFA assay. In this commercial production system, sows were vaccinated as pigs at 5 and 8 wk of age with Circumvent PCV (2 mL per dose; Intervet/Schering-Plough Animal Health, Millsboro, DE) and once with Circumvent PCV (2 mL dose) during gilt-development at 27 wk of age.

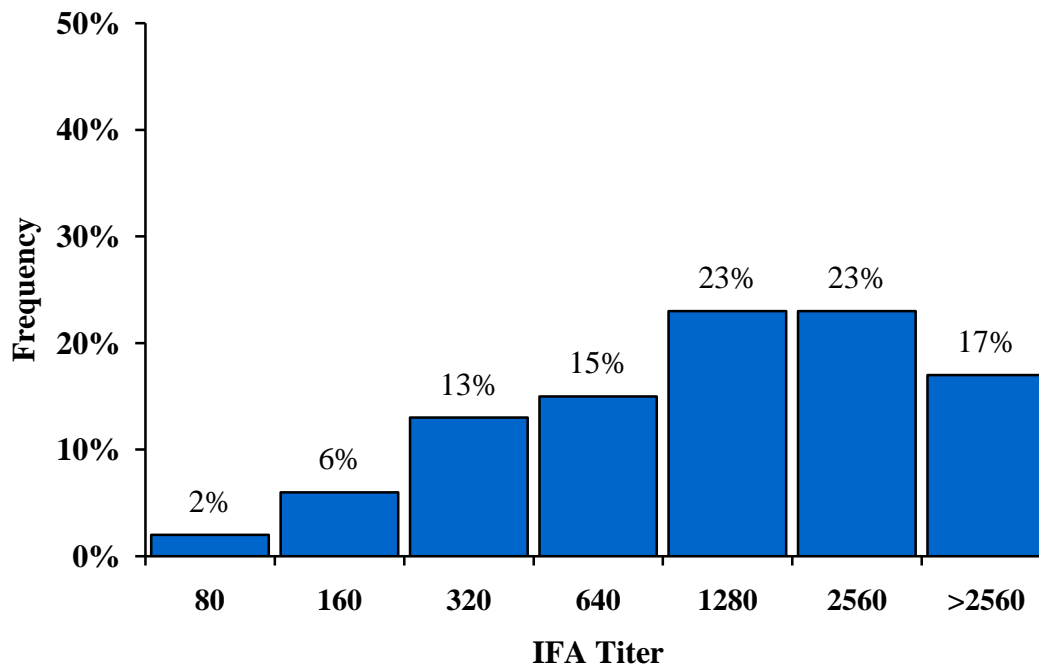


Figure 1.6 Indirect fluorescent antibody (IFA) geometric mean titers (GMT) of serum samples of weanling pigs tested over 6 days with 2 replications per sample (Exp. 3).

A total of 312 weanling pigs (146 barrows and 166 gilts) were sampled from 52 litters over 4 wk (13 litters per wk). On-farm and within laboratory factors were evaluated as sources of variation in PCV2 IFA testing. On-farm factors evaluated were gender (barrow or gilt) and weaning wk (1, 2, 3, or 4). Laboratory factors evaluated were IFA testing day (1, 2, 3, 4, 5, and 6) and sample replication. Replication indicated the dispensing order of serum (first dispense or second) from an electronic, programmable pipette for the initial dilution of the IFA testing procedure. Individual pig IFA titers were log₂ transformed and analyzed by analysis of variance using the GLIMMIX procedure in SAS version 9.1.3 (SAS Institute Inc., Cary, NC). The model used included fixed effects of gender, weaning wk, IFA testing day, replication, and all interactions. Litter was included as a random effect in the model. Resulting means were transformed back to the original scale for presentation as GMT.

IFA Day × Replication: $P = 0.03$

a,b,c,d,e $P < 0.05$

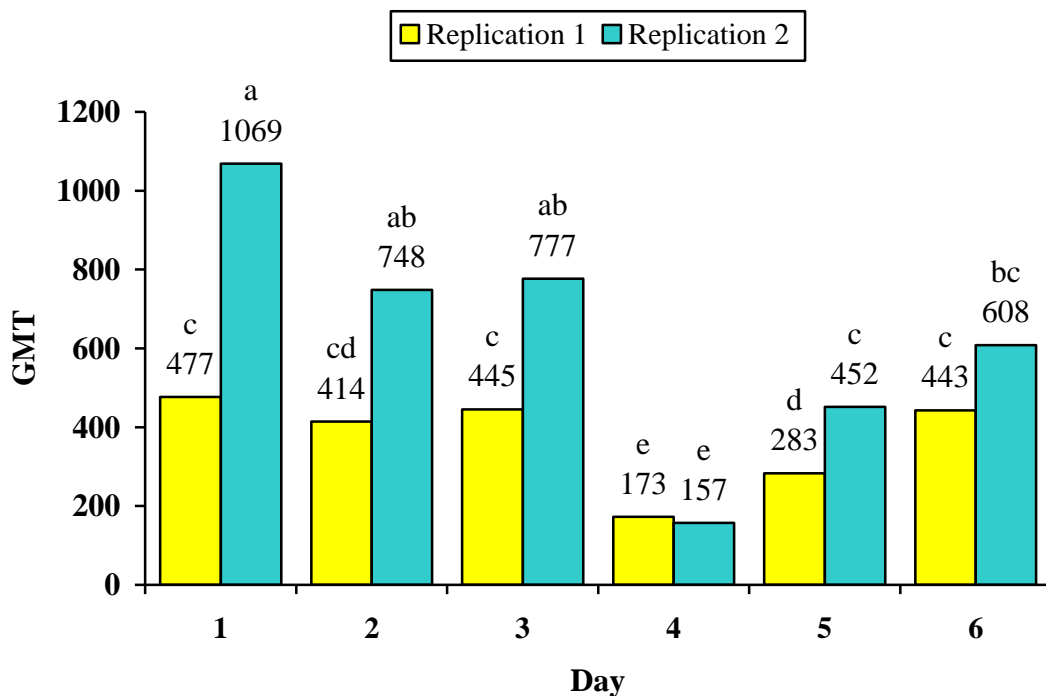


Figure 1.7 Indirect fluorescent antibody (IFA) geometric mean titers (GMT) of serum samples of weanling pigs collected over 4 wean weeks (Exp. 3).

A total of 312 weanling pigs (146 barrows and 166 gilts) were sampled from 52 litters over 4 wk (13 litters per wk). On-farm and within laboratory factors were evaluated as sources of variation in PCV2 IFA testing. On-farm factors evaluated were gender (barrow or gilt) and weaning wk (1, 2, 3, or 4). Laboratory factors evaluated were IFA testing day (1, 2, 3, 4, 5, and 6) and sample replication (first dispense or second dispense of serum from an electronic, programmable pipette during the dilution procedure). Individual pig IFA titers were \log_2 transformed and analyzed by analysis of variance using the GLIMMIX procedure in SAS version 9.1.3 (SAS Institute Inc., Cary, NC). The model used included fixed effects of gender, weaning wk, IFA testing day, replication, and all interactions. Litter was included as a random effect in the model. Resulting means were transformed back to the original scale for presentation as GMT.

Gender \times Week: $P = 0.06$

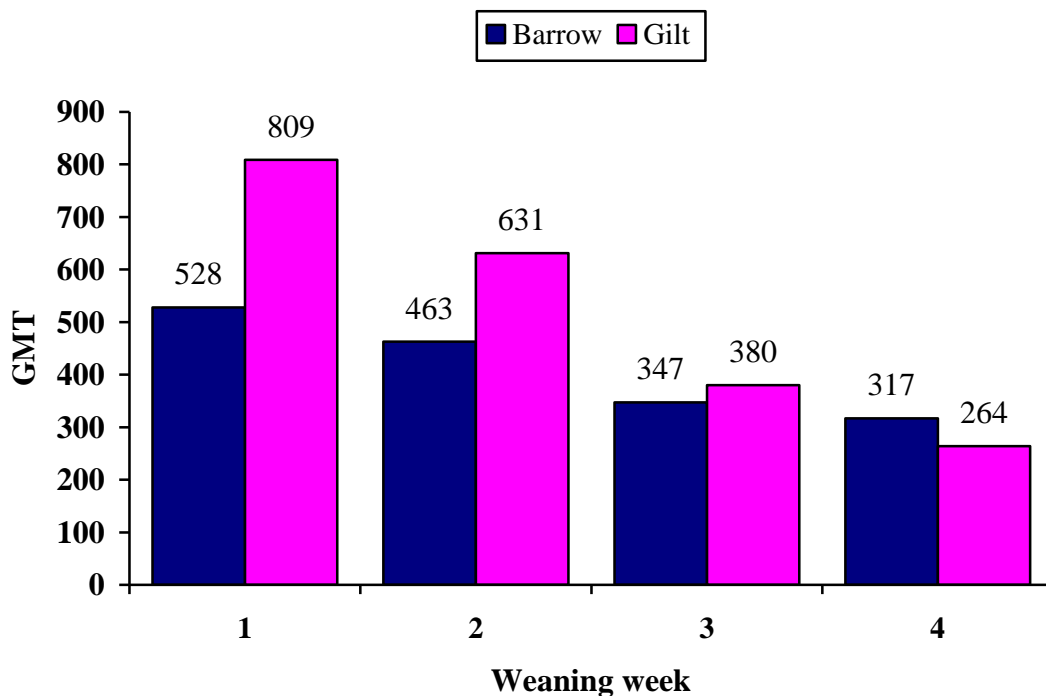


Table 1.1 Factors evaluated for their effects on pig indirect fluorescent antibody (IFA) testing (Exp. 3)

Fixed effect¹⁻⁴	F Value	Probability, <i>P</i> <
Gender × Week × IFA day × Replication	0.39	0.98
Gender × Week × IFA day	0.64	0.84
Gender × Week × Replication	0.16	0.92
Gender × IFA day × Replication	0.27	0.93
Week × IFA day × Replication	0.35	0.99
Gender × Week	2.44	0.06
Gender × IFA day	2.63	0.02
Week × IFA day	1.30	0.19
Gender × Replication	0.03	0.86
Week × Replication	0.17	0.92
IFA day × Replication	2.46	0.03
Gender	3.64	0.06
Week	1.64	0.19
IFA day	28.66	<0.001
Replication	28.70	<0.001

Note: The type 3 test of fixed effects in SAS version 9.1.3 (SAS Institute Inc., Cary, NC) was used to obtain these values.

¹ Gender was either barrow or gilt.

² Week indicates the wk of weaning for sampling.

³ IFA day indicates the day of IFA testing in the laboratory.

⁴ Replication indicates the order of serum loading (first dispense or second) for initial dilution when using an electronic, programmable pipette and running duplicate tests.

CHAPTER 2 - Genetic makeup influences pig growth rate responses to vaccination for porcine circovirus type 2

Summary

Objective(s): The objective of this field study was to compare the effect of vaccination for porcine circovirus type 2 (PCV2) on the growth rate of pigs with different genetic makeup in a high-health herd.

Materials and methods: A total of 454 weanling pigs (20.6 ± 1.98 d of age; 6.1 ± 1.27 kg BW) were used in a 130-d trial performed at a genetic multiplication farm in Kansas to determine the effect of circovirus vaccination on growth performance of boars and gilts of different genetic makeup. Genetic designations were: A×A (Duroc genotype), B×B (synthetic White Pietrain genotype), A×B, and B×A. Pigs were ranked by birth weight within litter and gender and randomly assigned to vaccination treatments (vaccinated or non-vaccinated control). Pigs allotted to the vaccinated treatment received 2 doses (2 mL per dose, intramuscular injection) of a circovirus vaccine (Circumvent PCV; Intervet/Schering-Plough Animal Health, Millsboro, DE) at 3 and 5 wk of age. Control pigs were comingled with vaccinated pigs throughout the trial. Serum was collected from all pigs on d 0 (weaning), d 40 (end of nursery period), and d 130 (off-test; approximately 150 d of age) to determine PCV2 viral load and antibody levels. Pigs were individually weighed at these time points to measure ADG. Measurements of loin and backfat depths were collected by real-time ultrasound at off-test. Data were analyzed on 417 pigs with complete growth records at off-test.

Results: Interactions ($P \leq 0.05$) between genetic makeup and vaccination treatment were observed for d 0 to 40, 40 to 130, and d 0 to 130 growth rates. On d 130, pigs vaccinated with the circovirus vaccine were heavier (genetic makeup-by-vaccination treatment interaction: $P = 0.05$) than controls. However, the magnitude of the mean weight difference between vaccinated and control pigs was almost 4 times greater in the A×A pigs than in the B×B pigs with A×B and B×A having intermediate weight differences. Thus, the growth response to circovirus vaccination was increased in the A×A population relative to the B×B population. Vaccination

reduced ($P < 0.001$) serum PCV2 viral load levels of vaccinated pigs compared with controls on both d 40 and d 130.

Implications: Pig genetic makeup affects growth rate response to circovirus vaccination and should be considered a risk factor for PCVD expression.

Key words: circovirus, genetics, growth, PCV2, vaccine

The etiologic agent of porcine circoviral disease (PCVD) (Allan and Ellis, 2000) is porcine circovirus type 2 (PCV2). Risk factors for the development of PCVD include concurrent viral or bacterial infections, management factors (Rose et al., 2003), gender, as well as genetics. In one study, Landrace pigs were at increased risk for developing clinical PCVD when compared with Duroc or Large White pigs (Opriessnig et al., 2006). In another study, there were differences in postweaning mortality between pigs from Pietrain, Large White \times Pietrain, and Large White \times Duroc lines with many mortalities having lesions consistent with PCVD (López-Soria et al., 2004). In contrast, yet another study failed to detect differences in PCVD-attributed mortality when comparing offspring sired by either Pietrain boars or boars less than 50% Pietrain (Rose et al., 2005). The results from these studies support speculation that varying degrees of genetic susceptibility to PCV2 infection or expression of PCVD may exist. Widespread availability of circovirus vaccines, documented to be effective in reducing mortality and increasing pig growth rate (Horlen et al., 2008), has promoted research efforts to determine the consistency and the magnitude of the effect from circovirus immunization. The limited reports of interaction between genetic background and PCVD expression provoke questions regarding the impact of response to circovirus immunization between differing genetic makeups. The focus of this study was to compare circovirus vaccination of two genetic populations and their crosses in a high-health herd using growth rate as a measure of vaccination response.

Materials and Methods

Procedures used in this field trial were approved by the Kansas State University Institutional Animal Care and Use Committee. This 130-d study was performed in a 1,700-sow genetic multiplication farm in Kansas. This farm was of a high-health status, being porcine

reproductive and respiratory syndrome virus negative and without evidence of *Mycoplasma hyopneumoniae* infection since stocking in 2000. In 2006, an increase in morbidity characterized by ill-thrift was observed. Histopathology lesions and gross clinical lesions consistent with PCVD were documented, and immunohistochemistry staining for PCV2 antigen and polymerase chain reaction (PCR) differentiation of the PCV2 genotype confirmed the presence of PCV2b.

This study utilized pigs born over a 7 d period. At birth, all pigs from 72 litters (662 pigs total) were weighed and identified by a unique ear-tag number. As this field study was performed in a boar multiplication farm, 128 pigs were eliminated because of low estimated breeding values or genetic defects. In addition, there were 57 pre-weaning deaths and 23 light-weight pigs which were held back at the time of weaning and thus not included in the study.

A total of 454 weanling pigs (20.6 ± 1.98 d of age; 6.1 ± 1.27 kg BW) representing 55 litters from the 4 genetic populations were ranked by birth weight within litter and gender (boar or gilt) and randomly assigned to vaccination treatments (vaccinated or non-vaccinated control). Genetic designations were: pure-lines of A×A (Duroc genotype) and B×B (synthetic White Pietrain genotype) and crossbreds A×B (Duroc sire × synthetic White Pietrain dam) and B×A (synthetic White Pietrain sire × Duroc dam). The circovirus vaccine administered to pigs assigned to the vaccinated treatment was a killed, 2-dose vaccine (Circumvent PCV, Intervet/Schering-Plough Animal Health, Millsboro, DE).

At weaning (d 0 of the trial) and again 2 wk later, pigs allotted to the vaccinated treatment were injected with the circovirus vaccine according to label dose and route of administration (2 mL per dose; IM injection). In this farm, farrowing and finishing facilities were managed all-in, all-out by room; nursery rooms were filled over 2 wk and all-out. All pigs on test were moved from the nursery rooms on d 40, were temporarily housed in a grower facility, and then moved to the finishing barn for the remainder of the trial. Vaccinated and non-vaccinated control pigs of the different genetic makeup were comingled in single-sex pens throughout the trial.

Serum was collected from all pigs on d 0, 40, and 130 to determine PCV2 viral load in serum and PCV2 antibody titers. Pigs were individually weighed at these time points to measure growth rate. Due to time constraints as pigs were weighed off test, 17% of the pigs (40 control pigs and 31 vaccinated pigs) with representation from all treatments were weighed on d 131. These 71 pigs had one additional day used in d 40 to 130 and d 0 to 130 growth rate calculations.

Backfat and loin depths, measured when pigs were weighed off test, were determined by real time ultrasound at the 10th rib P2 location.

Removals and deaths were recorded throughout the trial. There were 6 deaths from d 0 to 40 and 25 deaths from d 40 to 130. Other animals removed included 1 late castration, 2 with missing final weights, 1 with a final data entry error, 1 severe tail bite, and 1 downer pig. Statistical analyses were performed on individual records from 417 pigs which had complete growth records at the end of the trial.

Diagnostic testing:

Serum was stored in -80°C prior to indirect fluorescent antibody (IFA) and PCR testing. Diagnostic testing was performed at the Kansas State Veterinary Diagnostic Laboratory (KSVDL) after all samples had been collected. Diagnostic testing methods were accepted and validated in accordance with the American Association of Veterinary Laboratory Diagnosticians' standard requirements necessary for diagnostic laboratory accreditation. Serum samples were assayed for PCV2 antibodies using the 96-well format KSVDL PCV2 IFA assay with serial 1:2 dilutions beginning with a 1:20 serum to PBS dilution. Sample sets from control and vaccinated pigs were assayed simultaneously, and all samples from an individual pig were tested on the same IFA plate. Serum samples from d 40 and 130 were individually tested for PCV2 nucleic acid using the KSVDL PCV2 quantitative PCR assay. Extraction of PCV2 DNA and PCR testing was performed under similar laboratory conditions for individual control and vaccinated pig serum samples.

Analysis:

The effect of circovirus vaccination on growth response was determined by analysis of variance using the GLIMMIX procedure in SAS version 9.1.3 (SAS Institute Inc., Cary, NC) to obtain least squares means and standard errors for all response variables. The statistical model included the fixed effects of vaccination treatment, genetic makeup, gender, and all interactions. Litter of origin was included as a random effect. Response variables evaluated were days to weaning and off-test, birth weight, d 0 (weaning) weight, and d 130 (off-test) weight. Individual pig growth rate (ADG) was calculated by dividing the period weight gain by the number of days in the period. Growth rate period designations were: d 0 to 40 (nursery period), d 40 to 130 (grow-finish period), and d 0 to 130 (wean-to-finish period). Backfat and loin depths were

analyzed as unadjusted and adjusted values. For the adjusted analysis, backfat and loin depths were adjusted to a common average off-test body weight.

Significance tests were performed for comparisons of the means of fixed effect combinations and their interactions for all response criteria. Values of $P < 0.05$ were considered significant.

Prior to analysis, IFA titers were \log_2 transformed to approximate a normal distribution. For the IFA analysis, the \log_2 of 10 was used when samples did not have PCV2 antibody detected at the most concentrated dilution (1:20). The \log_2 of 5,120 was used when samples were strongly positive at the least concentrated dilution (1:2,560). These methods allowed those low antibody concentrated ($< 1:20$) or high antibody concentrated sample ($> 1:2,560$) results to be weighted differently than samples with normal-intensity fluorescence detected at dilutions of 1:20 and 1:2,560. The main and interactive effects of genetic makeup, vaccination treatment, gender, and time on IFA antibody responses were tested by repeated measures analysis using the MIXED procedure in SAS. The statistical model included the fixed effects of vaccination treatment, genetic makeup, gender, time, and all interactions. The resulting means were transformed back to the original scale for presentation as geometric mean titers.

Viral template quantities were \log_{10} transformed before analysis to achieve normality for the PCR data. Serum samples with any detectable PCV2 nucleic acid were considered to be PCV2 DNA-containing samples. Positive quantitative values were included in the analysis for these PCV2 DNA-containing samples along with zero-values from samples with no PCV2 DNA detected. The main and interactive effects of genetic makeup, vaccination treatment, and gender on d 40 and 130 serum PCV2 nucleic acid load were determined using the GLIMMIX procedure in SAS to obtain means and standard errors. Litter was included as a random effect for the analysis of PCR data. The resulting least squares means were transformed back to the original scale for presentation as geometric means.

The effect of d 40 PCV2 DNA template quantity on growth rate was determined using the GLIMMIX procedure in SAS. Fixed effects in the model included genetic makeup, vaccination treatment, d 40 \log_{10} transformed PCV2 template copies, and all interactions. Litter was included as a random effect. The solutions statement in SAS was used to determine intercepts and coefficients for the regression model.

Results

Mortality:

Postweaning mortality for the control pigs was 7.0% while the vaccinated pig mortality was 6.8% despite active PCV2 infection occurring during this trial. Statistical analysis was not performed on these data. However, there was no discernable difference in mortality between the genetic makeups.

Performance analysis:

There were no 3-way interactions between genetic makeup, vaccination treatment, and gender detected for any responses with the exception of weight-adjusted backfat depth ($P = 0.02$). This 3-way interaction was a result of control A×B boars having increased (11.9 ± 0.41 vs. 10.9 ± 0.41 mm, $P = 0.04$) backfat depth compared with vaccinated A×B boars. Within all other genetic makeup and gender combinations, backfat depth was similar ($P \geq 0.09$) between control and vaccinated pigs (data not shown).

There were no 2-way interactions ($P \geq 0.08$) observed between vaccination treatment and gender for any growth or carcass response criteria (data not shown). However, there were 2-way interactions between genetic makeup and vaccination treatment as well as with genetic makeup and gender (Table 2.1) for growth responses.

Age:

An interaction between genetic makeup and gender was observed for ages at d 0 ($P = 0.04$) and d 130 ($P < 0.001$). Resulting from an uneven birth pattern for boars and gilts within the A×A genetic makeup during the wk of farrowing, on d 0, A×A boars were 0.3 d younger (21.0 ± 0.43 vs. 21.3 ± 0.43 d, $P < 0.01$) than A×A gilts. There were no differences ($P \geq 0.40$) between ages of boars and gilts of B×B (boars: 19.6 ± 0.49 d; gilts: 19.7 ± 0.49 d), A×B (boars: 20.3 ± 0.47 d; gilts: 20.3 ± 0.47 d), and B×A (boars: 21.2 ± 0.65 d; gilts: 21.3 ± 0.65 d). The interaction at d 130 was a result of A×A boars being 0.2 d younger (151.4 ± 0.46 vs. 151.6 ± 0.46 d, $P < 0.01$) than A×A gilts, while B×B boars were 0.6 d younger (149.7 ± 0.52 vs. 150.3 ± 0.52 d, $P < 0.001$) than B×B gilts. There were no differences ($P \geq 0.55$) between d 130 ages of boars and gilts within A×B (boars: 150.6 ± 0.51 d; gilts: 150.6 ± 0.51 d) and B×A (boars: 151.7 ± 0.69 d; gilts: 150.7 ± 0.69 d). More importantly for evaluation of vaccination effects there was no interaction between genetic makeup and vaccination treatment ($P > 0.41$) or effect of vaccination ($P > 0.48$) observed for age at weaning or d 130.

Weight:

There were no interactions ($P \geq 0.24$) observed between genetic makeup and vaccination treatment or genetic makeup and gender for birth or d 0 weights. In addition, neither genetic makeup, vaccination treatment, nor gender affected ($P \geq 0.17$) birth or d 0 weights.

Weight on d 130 depended upon the 2-way interactions between genetic makeup and vaccination treatment ($P = 0.05$) as well as genetic makeup and gender ($P = 0.02$). Within A×A, gilts weighed less (93.1 ± 2.11 vs. 98.1 ± 2.09 kg BW, $P = 0.01$) compared with boars. Within B×B, A×B and B×A, boars and gilts had similar ($P \geq 0.07$) weights on d 130.

The 2-way interaction between genetic makeup and vaccination treatment was a result of A×A control pigs being lighter ($P < 0.001$) than vaccinated A×A pigs on d 130 whereas weights of control and vaccinated pigs of B×B, A×B, and B×A were similar ($P \geq 0.06$). Regardless, within all genetic populations, mean weights of control pigs on d 130 were numerically less than those of vaccinated pigs. Vaccination increased weight compared with that of controls within the A×A population (vaccinated pig mean BW: 100.1 kg vs. control pig mean BW: 91.1 kg; 9.0 kg improvement due to vaccination) almost 4 times that of the effect within B×B population (vaccinated pig mean BW: 102.4 kg vs. control pig mean BW: 100.1 kg; 2.3 kg improvement due to vaccination). The vaccination effect on growth rate in the crossbred pigs was intermediate to that of pure-lines (A×A and B×B).

The distribution of off-test weights for control and vaccinated pigs within A×A (Figure 2.1) and B×B (Figure 2.2) were determined. These distributions demonstrated the right-shift in the d 130 weights of the vaccinated pig population relative to the control pigs. The magnitude of the mean weight difference between the control and vaccinated pigs was almost 4 times greater in the A×A pigs (9.0 kg difference) compared with the B×B pigs (2.3 kg difference). Demonstrated by the population shift within both distributions, vaccination affected the entire vaccinated pig population though the extent of the effect of vaccination was different within each genetic population.

Average daily gain:

There was no interactive effect ($P = 0.22$) between genetic makeup and gender on d 0 to 40 ADG; however, gender did affect ($P < 0.001$) growth rate during this period. From d 0 to 40, gilts grew faster (430 ± 9.4 vs. 403 ± 9.2 g per day) than boars.

An interactive effect ($P = 0.05$) was detected between genetic makeup and vaccination treatment for d 0 to 40 ADG. There was a crossing-interaction for growth rate of control and vaccinated pigs within the A×B and B×A populations. Control pigs of A×B had greater ADG compared with A×B vaccinated pigs ($P = 0.04$), while B×A vaccinated pigs had numerically greater ADG compared to B×A controls ($P = 0.11$).

There were 2-way interactive effects between both genetic makeup and gender ($P = 0.03$) and genetic makeup and vaccination treatment ($P = 0.05$) detected for d 40 to 130 ADG. Boars of A×A grew faster (845 ± 17.6 vs. 790 ± 17.8 g per day, $P < 0.01$) than A×A gilts whereas boars and gilts grew at a similar ($P \geq 0.15$) rate within B×B, A×B, and B×A. From d 40 to 130, ADG was decreased ($P \leq 0.001$) for the A×A controls compared with the growth rates of the control pigs of A×B, B×A, and B×B. Controls from the latter 3 genetic populations had similar ($P \geq 0.45$) d 40 to 130 ADG. In contrast, A×A vaccinated pigs had similar ($P \geq 0.14$) ADG compared with vaccinated pigs from the other genetic makeups with the exception of vaccinated A×B pigs ($P = 0.04$). Thus, the difference between vaccinated and control pig mean growth rate was greater within A×A compared with the difference within B×B, A×B, and B×A.

There were interactive effects between genetic makeup and gender ($P = 0.03$) and genetic makeup and vaccination treatment ($P = 0.04$) observed for d 0 to 130 ADG. Boars of A×A grew faster (705 ± 15.2 vs. 671 ± 15.3 g per day, $P = 0.02$) than gilts of A×A whereas within B×B, A×B, and B×A, boars and gilts had similar ($P \geq 0.06$) d 0 to 130 growth rates. Growth rate of A×A control pigs was reduced ($P \leq 0.002$) compared with control pigs from the other genetic makeups. Vaccinated A×A pigs grew slower ($P = 0.04$) when compared with B×A vaccinated pigs, but had similar ($P \geq 0.06$) ADG with vaccinated A×B and B×B pigs. These differences in d 0 to 130 growth rates resulted in the magnitude of the difference between vaccinated and control pig mean growth rates being greatest in pigs of A×A.

Backfat and loin depth:

There were no 3-way or 2-way interactive effects ($P \geq 0.05$) observed for unadjusted backfat depth. Genetic makeup did affect ($P = 0.02$) backfat depth as A×A (11.7 ± 0.29 mm) and A×B (12.0 ± 0.30 mm) pigs had increased ($P \leq 0.02$) backfat depth compared with B×B pigs (10.7 ± 0.32 mm). Backfat depth of B×A (11.5 ± 0.42 mm) pigs was intermediate ($P \geq 0.14$) to measurements of A×A and B×B pigs.

There were no 3-way or 2-way interactive effects ($P \geq 0.10$) detected for unadjusted loin depth; however, loin depth was affected ($P < 0.001$) by genetic makeup, vaccination treatment, and gender. Loin depth of A×A pigs was decreased (60.7 ± 0.75 vs. 66.3 ± 0.77 mm, $P < 0.001$) compared with that of A×B pigs. Pigs of A×B had similar ($P = 0.28$) loin depth compared with B×A pigs (67.7 ± 1.06 mm) and increased ($P < 0.01$) loin depth compared with B×B pigs (69.2 ± 0.81 mm). Control pigs had decreased (65.0 ± 0.50 vs. 66.9 ± 0.51 mm; $P < 0.01$) loin depth compared with vaccinated pigs, while boars had decreased (63.9 ± 0.50 vs. 68.0 ± 0.52 mm; $P < 0.01$) loin depth compared with gilts.

As previously explained, after backfat depth measurements were adjusted to a common average d 130 weight, there was a 3-way interaction ($P = 0.02$) observed with genetic makeup, gender, and vaccination treatment. This interaction ($P = 0.02$) was the result of control A×B boars having increased (11.9 ± 0.41 vs. 10.9 ± 0.41 mm; $P = 0.04$) weight-adjusted backfat depth compared with vaccinated A×B boars. Within all other gender by genetic makeup combinations, backfat depth was similar ($P \geq 0.09$) between control and vaccinated pigs. The interactive effects ($P = 0.79$) between genetic makeup and vaccination treatment for weight-adjusted backfat depth are presented in Figure 2.3.

After loin depths were adjusted to a common off-test weight, there was no significant 3-way interaction ($P = 0.71$) observed. There was a 2-way interaction ($P = 0.01$) detected between genetic makeup and gender. Despite loin depths of gilts consistently being increased compared with boars, within the A×B makeup the difference was 2.2 mm whereas within A×A, B×B, and B×A the difference was 4.3 mm or greater. Although there was a significant effect of vaccination treatment prior to weight-adjustment, after adjustment to a common average d 130 weight, vaccination treatment failed ($P = 0.29$) to affect loin depth. The interactive effect ($P = 0.82$) of genetic makeup and vaccination treatment for weight-adjusted loin depth are presented in Figure 2.4.

Indirect fluorescent antibody results:

The IFA geometric mean titers for control and vaccinated pigs on d 0, 40, and 130 are presented in Figure 2.5. These profiles indicate the timing of the PCV2 antibody response due to vaccination with the 2-dose circovirus vaccine in contrast with the antibody response produced from natural PCV2 exposure.

There were 3-way interactions with genetic makeup, vaccination treatment, and time ($P = 0.02$), as well as with vaccination treatment, gender, and time ($P < 0.01$) detected for IFA antibody response. Vaccinated pigs demonstrated an increase in PCV2 antibody titer by d 40 which decreased by d 130, while control pigs did not have a rise in PCV2 antibody level detected until d 130.

Polymerase chain reaction results:

There were no 3-way or 2-way interactions ($P \geq 0.15$) with genetic makeup, vaccination treatment, or gender on d 40 or d 130 PCV2 viral template copy quantity.

Viral template copy quantity was affected by both genetic makeup ($P < 0.01$, Figure 2.6) and vaccination treatment ($P < 0.001$). On d 40, B×B pigs had a decreased (56.8 template copies per reaction, $P \leq 0.02$) PCV2 DNA load compared with pigs of A×A, A×B, and B×A (420.1 template copies per reaction or higher). Vaccinated pigs also had decreased (20.9 viral copies per reaction vs. 4582.5 viral copies per reaction, $P < 0.001$) PCV2 viral template copy quantities compared with control pigs (Figure 2.7).

Only vaccination treatment affected ($P < 0.001$) d 130 PCV2 viral template copy quantity. Vaccinated pigs had decreased serum viral template quantities (1.3 viral template copies per reaction vs. 3.8 viral template copies per reaction) compared with control pigs (Figure 2.7).

There was a 3-way interaction ($P = 0.04$) with genetic makeup, vaccination treatment, and d 40 PCV2 DNA template quantity observed for d 40 to 130 ADG. Growth rate from d 40 to 130 depended not only on both genetic makeup and vaccine status but also on d 40 viral load. As viral load increased, the growth rate response differed depending on the genetic makeup and vaccination status. Growth rate was modeled with the \log_{10} transformed d 40 PCR data and, to demonstrate the disparity in growth rates, the models for A×A controls and B×B controls are included (Figure 2.8).

Discussion

Porcine circovirus disease is a devastating disease affecting multiple organ systems. Infection with PCV2 has become endemic in many swine herds. The virus itself is ubiquitous, present in nearly all herds, yet the expression of disease (PCVD) pertaining to morbidity and mortality varies. Factors associated with the risk for development of PCVD disease or lesions

have been identified including genetics (Opriessnig et al., 2006), gender (Corr  ge et al., 2001), litter of origin (Madec et al., 2000), low birth or weaning weight (Corr  ge et al., 2001), and management factors (Rose et al., 2003). Though PCV2 is the necessary etiologic agent of PCVD, there is evidence that clinical disease is exacerbated when accompanied by additional pathologic agents, also called cofactors (Dorr et al., 2007; Opriessnig et al., 2004). In general, circovirus vaccines have been effective in lessening the severity of or preventing clinical PCVD (Desrosiers et al., 2009; Kixm  ller et al., 2008). In development of vaccination programs as standard practice for many farms, understanding vaccine limitations and expected responses to vaccination has become a focus within the industry.

Results from our study indicate that growth rate response to circovirus vaccination varies with genetic makeup. To our knowledge, this is the first study demonstrating differential responses to circovirus vaccination based on genetic makeup under field conditions. Genetic background, however, had been previously implicated by other researchers as a risk factor for expression of PCVD (Opriessnig et al., 2006; Opriessnig et al., 2009b). Our study supports the evidence that PCVD risk is dependent on the genetic makeup of the pig.

Field reports have suggested pigs from Pietrain background may be less susceptible to PCVD (L  pez-Soria et al., 2004); however, some published study results do not support these observations (Rose et al., 2005). In our study, the magnitude of the response was greater in pigs of the Duroc genotype compared to pigs of the White Pietrain genotype. Also, our research adds to the body of literature indicating that pig genotype affects responses to vaccination or disease expression (Doeschl-Wilson et al., 2009; Reiner et al., 2002; Vincent et al., 2006).

Performing this study in a high-health status herd with few clinical signs of PCVD other than an increase in morbidity provides unique insight on PCVD to the current literature. Previous research, evaluating host genetics as a risk factor, has focused on documenting the effects of genetic makeup on PCVD-associated mortality, observed clinical disease with wasting, or PCV2-lesion severity differences (L  pez-Soria et al., 2004; Opriessnig et al., 2006; Opriessnig et al., 2009b; Rose et al., 2005). Mortality had not increased in the herd used for our study, thus mortality was not a primary response of interest. Nonetheless, mortalities were recorded and there was only a 0.2% difference between control and vaccinated pig mortality percentages.

The effect of circovirus vaccination on growth rate from d 0 to 130 for pigs of A  A was about 4 times greater (A  A: 66 g per day vs. B  B: 16 g per day) than the effect of vaccination

on growth rate for pigs of B×B. Although the magnitude of the difference between controls and vaccinates was greater in the A×A pigs than in the B×B pigs, the control and vaccinate response patterns within the genetic makeups were similar. Evident from weight distributions for both pure-line populations, off-test weights (Figures 2.1 and 2.2) were shifted to the right for the vaccinated pigs compared with the control pigs. It was apparent that all pigs in both populations were affected by vaccination, with only the extent of the response being different. These findings support previous reports that circovirus vaccine affected all pigs, even heavy pigs (Horlen et al., 2008; Madec et al., 2000). Detection of the more pronounced right-shift for the vaccinated pigs within the weight distribution of the A×A population than in the B×B population provided new evidence that genetic makeup affects this circovirus vaccine response.

Similar to the results from another study (Jacela et al., 2011), vaccination against circovirus had little or no influence on carcass composition in our study. The loin depth differences detected initially between pigs of different vaccination treatments resulted from differences in d 130 weights. After weight adjustment to a common off-test weight, no difference was detected between loin depths across vaccination treatment.

Presence of PCV2 virus and active infection was confirmed during this trial by IFA and PCR testing. The IFA results indicate a PCV2 antibody rise in the vaccinated pigs by d 40. By d 130, antibody levels in the vaccinated pigs had decreased suggesting the increase by d 40 was primarily due to vaccination and not early natural viral exposure. In contrast, the rise in antibody titer between d 40 and 130 for the control pigs indicated natural PCV2 exposure had occurred.

Active circovirus infection was documented with detection of PCV2 DNA in serum of both control and vaccinated pigs. Viremia was documented by the end of the 40 d nursery phase with some pigs still PCV2-viremic on d 130. Although some nursery pigs were PCV2-viremic, the rise in IFA titer after d 40 suggests that many control pigs seroconverted after being moved from the nursery. These results indicate PCV2 circulation and infection during the nursery period with subsequent transmission or persistence during the finishing period. Regardless, control and vaccinated pigs were comingled during both the d 0 to 40 and d 40 to 130 periods and at both d 40 and 130, PCV2 viral load in vaccinated pigs was markedly decreased compared with that of control pigs.

Under experimental conditions, histopathologic lesion severity, particularly in liver and lymphoid tissues, worsened with increased PCV2 viral load detected by immunohistochemistry

(Krakowka et al., 2005). This trial provided data which suggests that growth rate depends upon serum viral load. Pure-line (A×A and B×B) control pig results indicate as d 40 viral load increased, d 40 to 130 growth rate decreased; however, the rate at which the change occurred was dependent upon genetic makeup. Growth rate decreased at a faster rate as viral load increased for control pigs of A×A compared with controls of the B×B makeup. Further research is needed to comprehensively explain the effects of serum viral load level as it relates to performance. Our data provided initial information linking serum viral load and growth performance; however, the significance biologically has yet to be fully characterized. The results of this study confirm previous research findings that circovirus vaccination effectively decreases serum viral load (Fachinger et al., 2008; Horlen et al., 2008; Opriessnig et al., 2009a), even under conditions where vaccinated pigs were housed with control pigs.

Genetic makeup also affected d 40 PCV2 viral loads. Pigs of B×B genetic makeup had the lowest level of PCV2 DNA detected on d 40. This genetic population also had the smallest response to circovirus vaccination as measured by growth rate. While these findings support observations that growth rate was negatively affected by increasing viral load, they also provide evidence that genetic makeup may play a role in the PCV2-infection susceptibility or resistance.

McIntosh et al. (2006) documented breed differences in PCV2 shedding duration. A survey of PCV2-antibody positive boars in a commercial boar stud revealed detectable PCV2 DNA in semen from Duroc and Landrace boars. Throughout the sampling time-frame, there was no PCV2 DNA detected in semen from Hamline, Large White maternal or paternal lines, or Meishan-synthetic breeds (McIntosh et al., 2006). While pig genotype has been shown to affect expression of other viral diseases (Halbur et al., 1998; Petry et al., 2005; Reiner et al., 2002), McIntosh et al. (2006) suggested that genetic makeup may be important for explaining differences in PCV2-infection susceptibility and expression.

Although the responses observed in our study were attenuated by circovirus vaccination, these data may indicate that genetic makeup affects PCVD expression. If the severity of PCV2 infection was dependent at some level on host genetics, this might explain why vaccination affected the genetic makeups differently. The B×B population was of a White Pietrain genotype and the magnitude of the growth difference between control and vaccinated pigs was less than that of the A×A pigs of a Duroc genotype. These findings resulted in additional questions regarding differences in susceptibility between these genetic makeups and whether it might be

possible to derive animals with improved circovirus-induced disease resistance. However, the primary objective of this trial was to determine whether pigs of different genetic makeups differed in their response to circovirus vaccination. With that difference clearly demonstrated, further research is needed to address these additional questions.

Results of this study indicate that pig genetic makeup affects the growth rate response to circovirus vaccination. Thus, pig genetic makeup must be considered as a risk factor relative to expression of PCVD and when evaluating performance responses to circovirus vaccination.

Implications

- Different genetic makeups respond differently to circovirus vaccination as measured by growth rate.
- This field trial further supports findings of reduced PCV2 viral load levels and improved growth rate in vaccinated pigs compared with non-vaccinated control pigs.
- Genetic makeup should be considered as a risk factor for either PCVD expression or response to circovirus vaccine.

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Figure 2.1 Distribution of pig weights at off-test for control versus vaccinated pigs of genetic makeup A×A (Duroc genotype).

A total of 454 weanling pigs from 4 genetic populations were ranked by birth weight within litter and gender (boar or gilt) and randomly assigned to vaccination treatments (vaccinated or non-vaccinated control). Genetic designations were: pure-lines of A×A (Duroc genotype) and B×B (synthetic White Pietrain genotype) and crossbreds A×B and B×A. Pigs allotted to the vaccinated treatment were injected (2 mL per dose) at weaning and again 2 wk later with a circovirus vaccine (Circumvent PCV, Intervet/Schering-Plough Animal Health, Millsboro, DE). Pigs were weighed on d 0 (weaning), d 40 (end-of-nursery), and approximately d 130 (off-test; approximately 150 d of age). Individual pig records from 417 pigs that had complete growth records were used for analysis.

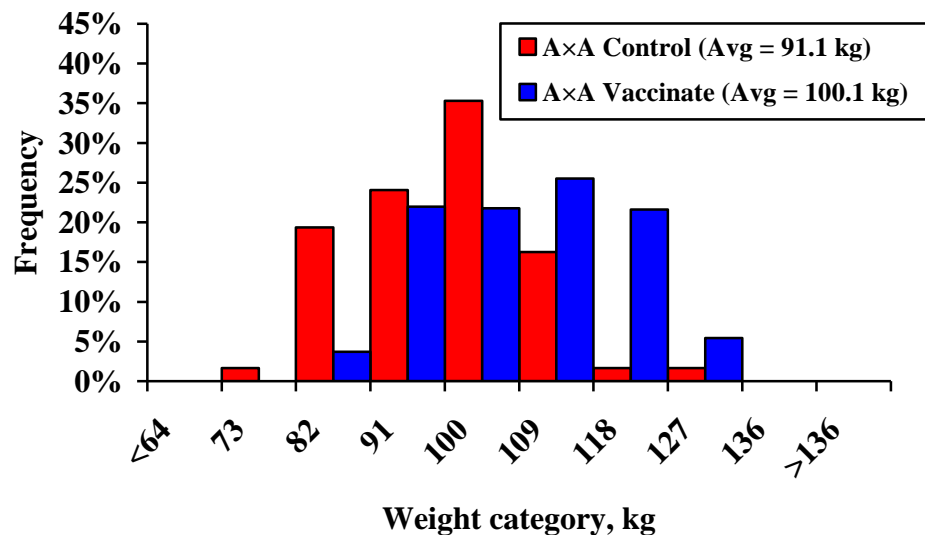


Figure 2.2 Distribution of pig weights at off-test for control versus vaccinated pigs of genetic makeup B×B (synthetic White Pietrain genotype).

A total of 454 weanling pigs from 4 genetic populations were ranked by birth weight within litter and gender (boar or gilt) and randomly assigned to vaccination treatments (vaccinated or non-vaccinated control). Genetic designations were: pure-lines of A×A (Duroc genotype) and B×B (synthetic White Pietrain genotype) and crossbreds A×B and B×A. Pigs allotted to the vaccinated treatment were injected (2 mL per dose) at weaning and again 2 wk later with a circovirus vaccine (Circumvent PCV, Intervet/Schering-Plough Animal Health, Millsboro, DE). Pigs were weighed on d 0 (weaning), d 40 (end-of-nursery), and approximately d 130 (off-test; approximately 150 d of age). Individual pig records from 417 pigs that had complete growth records were used for analysis.

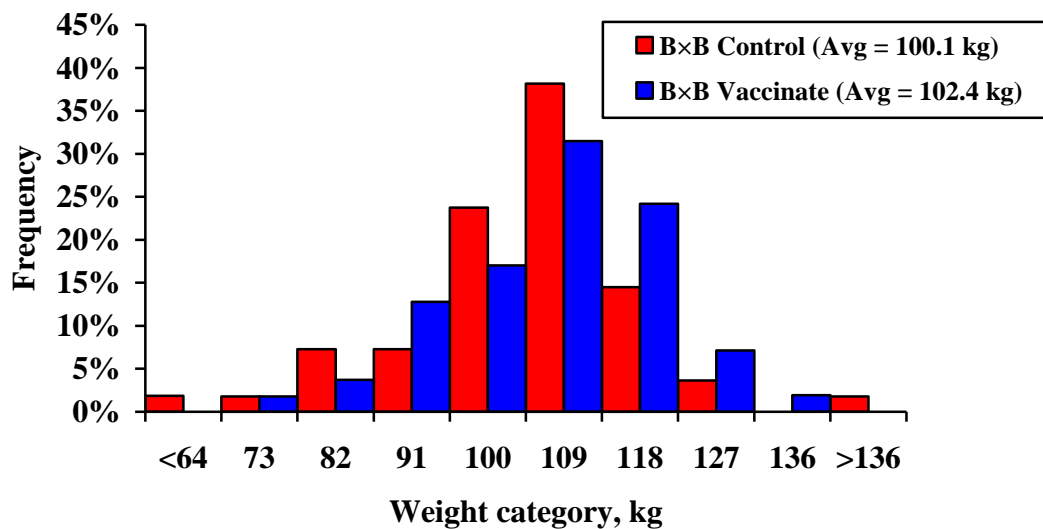


Figure 2.3 Weight-adjusted backfat depth for control and vaccinated pigs of different genetic makeups.

A total of 454 weanling pigs from 4 genetic populations were ranked by birth weight within litter and gender (boar or gilt) and randomly assigned to vaccination treatments (vaccinated or non-vaccinated control). Genetic designations were: pure-lines of A×A (Duroc genotype) and B×B (synthetic White Pietrain genotype) and crossbreds A×B and B×A. Pigs allotted to the vaccinated treatment were injected (2 mL per dose) at weaning and again 2 wk later with a circovirus vaccine (Circumvent PCV, Intervet/Schering-Plough Animal Health, Millsboro, DE). Pigs were weighed on d 0 (weaning), d 40 (end-of-nursery), and approximately d 130 (off-test; approximately 150 d of age). At off-test, backfat and loin depths were measured by real time ultrasound at the 10th rib P2 location. Individual pig records from 417 pigs that had complete growth records at off-test were analyzed by analysis of variance using the GLIMMIX procedure in SAS version 9.1.3 (SAS Institute Inc., Cary, NC). The model included the fixed effects of vaccination treatment, genetic makeup, gender, and all interactions. Litter of origin was included as a random effect. Backfat and loin depths were analyzed as unadjusted and adjusted (adjusted to a common average off-test body weight) values.

Genetic makeup × Vaccination treatment × Gender: $P = 0.02$

Genetic makeup × Vaccination treatment: $P = 0.79$

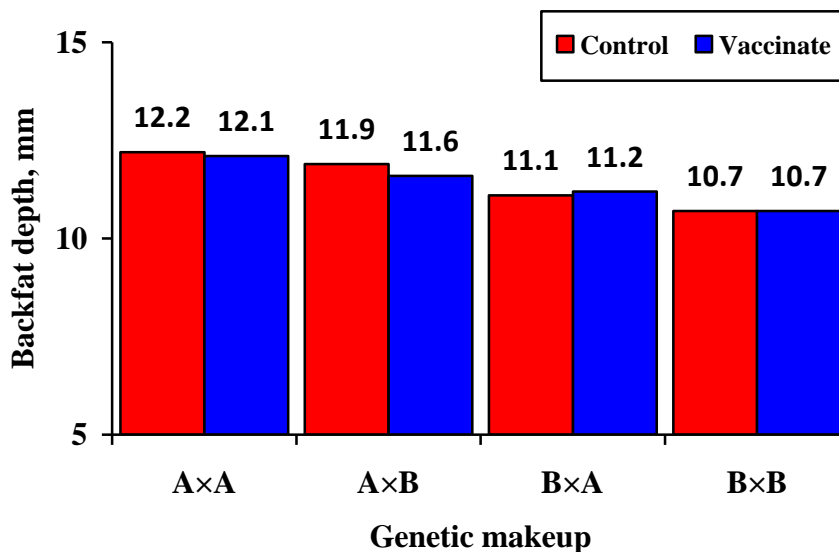


Figure 2.4 Weight-adjusted loin depth for control and vaccinated pigs of different genetic makeups.

A total of 454 weanling pigs from 4 genetic populations were ranked by birth weight within litter and gender (boar or gilt) and randomly assigned to vaccination treatments (vaccinated or non-vaccinated control). Genetic designations were: pure-lines of A×A (Duroc genotype) and B×B (synthetic White Pietrain genotype) and crossbreds A×B and B×A. Pigs allotted to the vaccinated treatment were injected (2 mL per dose) at weaning and again 2 wk later with a circovirus vaccine (Circumvent PCV, Intervet/Schering-Plough Animal Health, Millsboro, DE). Pigs were weighed on d 0 (weaning), d 40 (end-of-nursery), and approximately d 130 (off-test; approximately 150 d of age). At off-test, backfat and loin depths were measured by real time ultrasound at the 10th rib P2 location. Individual pig records from 417 pigs that had complete growth records at off-test were analyzed by analysis of variance using the GLIMMIX procedure in SAS version 9.1.3 (SAS Institute Inc., Cary, NC). The model included the fixed effects of vaccination treatment, genetic makeup, gender, and all interactions. Litter of origin was included as a random effect. Backfat and loin depths were analyzed as unadjusted and adjusted (adjusted to a common average off-test body weight) values.

Genetic makeup × Vaccination treatment: $P = 0.82$

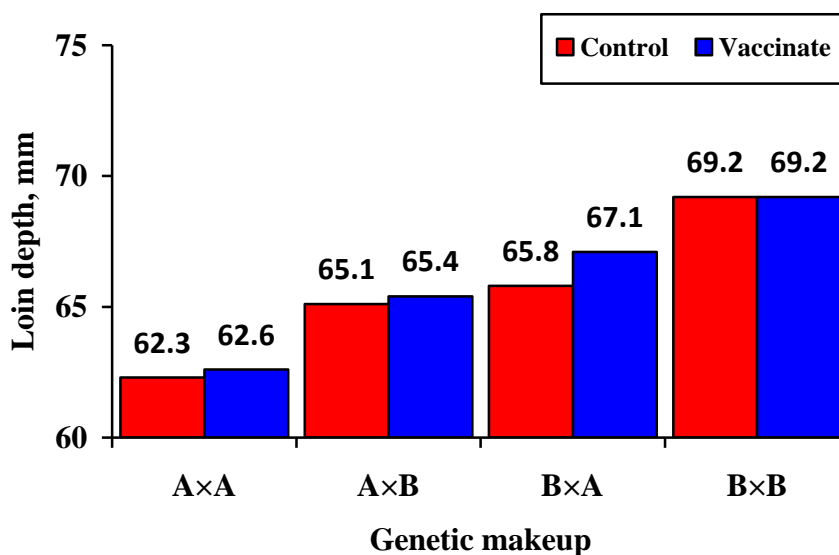


Figure 2.5 Indirect fluorescent antibody (IFA) geometric mean titers (GMT) on d 0, 40, and 130 for control and vaccinated pigs.

A total of 454 weanling pigs from 4 genetic populations were ranked by birth weight within litter and gender (boar or gilt) and randomly assigned to vaccination treatments (vaccinated or non-vaccinated control). Genetic designations were: pure-lines of A×A (Duroc genotype) and B×B (synthetic White Pietrain genotype) and crossbreds A×B and B×A. Pigs allotted to the vaccinated treatment were injected (2 mL per dose) at weaning and again 2 wk later with a circovirus vaccine (Circumvent PCV, Intervet/Schering-Plough Animal Health, Millsboro, DE). Serum was collected from pigs on d 0 (weaning), d 40 (end-of-nursery), and approximately d 130 (off-test; approximately 150 d of age). Statistical analyses were performed on data from 417 pigs which had complete growth records at off-test. Individual pig IFA data were log₂ transformed and then were analyzed by repeated measures analysis of variance using the MIXED procedure in SAS version 9.1.3 (SAS Institute Inc., Cary, NC). The model included the fixed effects of vaccination treatment, genetic makeup, gender, time, and all interactions. Resulting means were transformed back to the original scale for presentation.

Genetic makeup × Vaccination treatment × Time: $P = 0.02$

Vaccination treatment × Gender × Time: $P < 0.01$

Vaccination treatment × Time: $P < 0.01$

^{a,b,c,d} $P < 0.01$

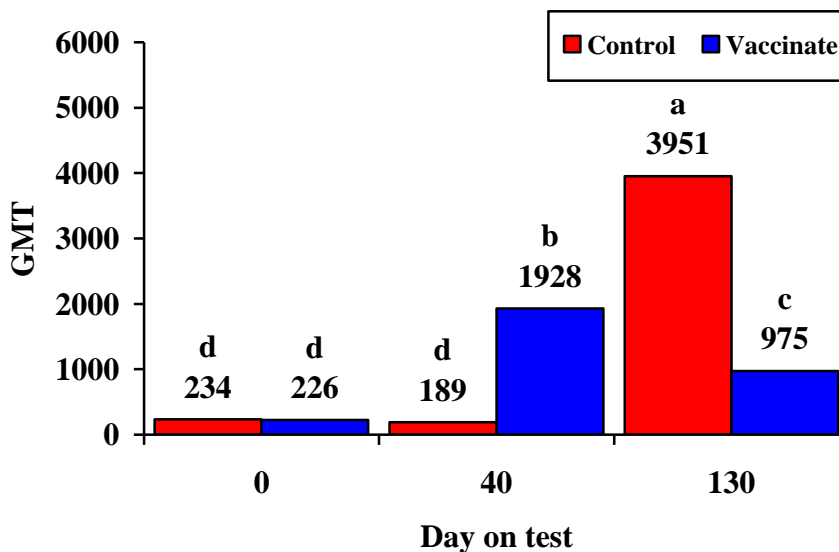


Figure 2.6 Porcine circovirus type 2 (PCV2) viral template quantity determined by PCV2 polymerase chain reaction (PCR) in serum collected on d 40 from control and vaccinated pigs of different genetic makeups.

A total of 454 weanling pigs from 4 genetic populations were ranked by birth weight within litter and gender (boar or gilt) and randomly assigned to vaccination treatments (vaccinated or non-vaccinated control). Genetic designations were: pure-lines of A×A (Duroc genotype) and B×B (synthetic White Pietrain genotype) and crossbreds A×B and B×A. Pigs allotted to the vaccinated treatment were injected (2 mL per dose) at weaning and again 2 wk later with a circovirus vaccine (Circumvent PCV, Intervet/Schering-Plough Animal Health, Millsboro, DE). Serum was collected from pigs on d 0 (weaning), d 40 (end-of-nursery), and approximately d 130 (off-test; approximately 150 d of age). Statistical analyses were performed on data from 417 pigs which had complete growth records at off-test. Individual pig PCV2 PCR data were log₁₀ transformed and then were analyzed by analysis of variance using the GLIMMIX procedure in SAS version 9.1.3 (SAS Institute Inc., Cary, NC). The model included the fixed effects of vaccination treatment, genetic makeup, gender, and all interactions. Litter of origin was included as a random effect. Resulting means were transformed back to the original scale for presentation.

Genetic makeup: $P < 0.01$

^{a,b} $P < 0.02$

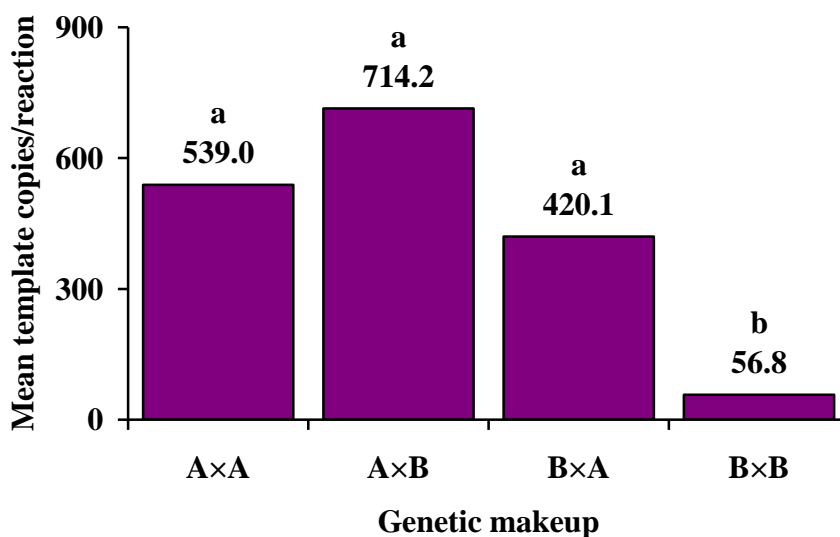


Figure 2.7 Porcine circovirus type 2 (PCV2) viral template quantity determined by PCV2 polymerase chain reaction (PCR) of serum samples collected on d 40 and 130 from control and vaccinated pigs.

A total of 454 weanling pigs from 4 genetic populations were ranked by birth weight within litter and gender (boar or gilt) and randomly assigned to vaccination treatments (vaccinated or non-vaccinated control). Genetic designations were: pure-lines of A×A (Duroc genotype) and B×B (synthetic White Pietrain genotype) and crossbreds A×B and B×A. Pigs allotted to the vaccinated treatment were injected (2 mL per dose) at weaning and again 2 wk later with a circovirus vaccine (Circumvent PCV, Intervet/Schering-Plough Animal Health, Millsboro, DE). Serum was collected from pigs on d 0 (weaning), d 40 (end-of-nursery), and approximately d 130 (off-test; approximately 150 d of age). Statistical analyses were performed on data from 417 pigs which had complete growth records at off-test. Individual pig PCV2 PCR data were log₁₀ transformed and then were analyzed by analysis of variance using the GLIMMIX procedure in SAS version 9.1.3 (SAS Institute Inc., Cary, NC). The model included the fixed effects of vaccination treatment, genetic makeup, gender, and all interactions. Litter of origin was included as a random effect. Resulting means were transformed back to the original scale for presentation.

^{a,b} $P < 0.001$ within day

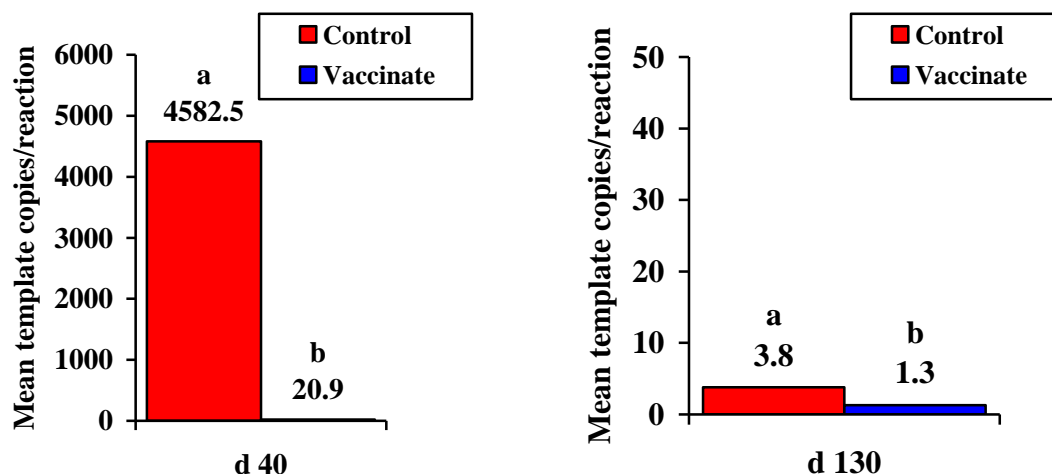


Figure 2.8 Effect of genetic makeup, circovirus vaccination, and d 40 serum PCV2 viral template quantity on d 40 to 130 average daily gain (ADG).

A total of 454 weanling pigs from 4 genetic populations were ranked by birth weight within litter and gender (boar or gilt) and randomly assigned to vaccination treatments (vaccinated or non-vaccinated control). Genetic designations were: pure-lines of A×A (Duroc genotype) and B×B (synthetic White Pietrain genotype) and crossbreds A×B and B×A. Pigs allotted to the vaccinated treatment were injected (2 mL per dose) at weaning and again 2 wk later with a circovirus vaccine (Circumvent PCV, Intervet/Schering-Plough Animal Health, Millsboro, DE). Individual pigs were weighed and serum was collected on d 0 (weaning), d 40 (end-of-nursery), and approximately d 130 (off-test; approximately 150 d of age). Individual pig d 40 PCV2 PCR data were log₁₀ transformed before analysis. The effect of d 40 PCV2 DNA template quantity on growth rate was determined using the GLIMMIX procedure in SAS version 9.1.3 (SAS Institute Inc., Cary, NC). Fixed effects in the model included genetic makeup, vaccination treatment, d 40 log₁₀ transformed PCV2 template copies, and all interactions. Litter was included as a random effect. The solutions statement in SAS was used to determine intercepts and coefficients for the regression model.

Genetic makeup × Vaccination treatment × PCRLLog: $P = 0.04$

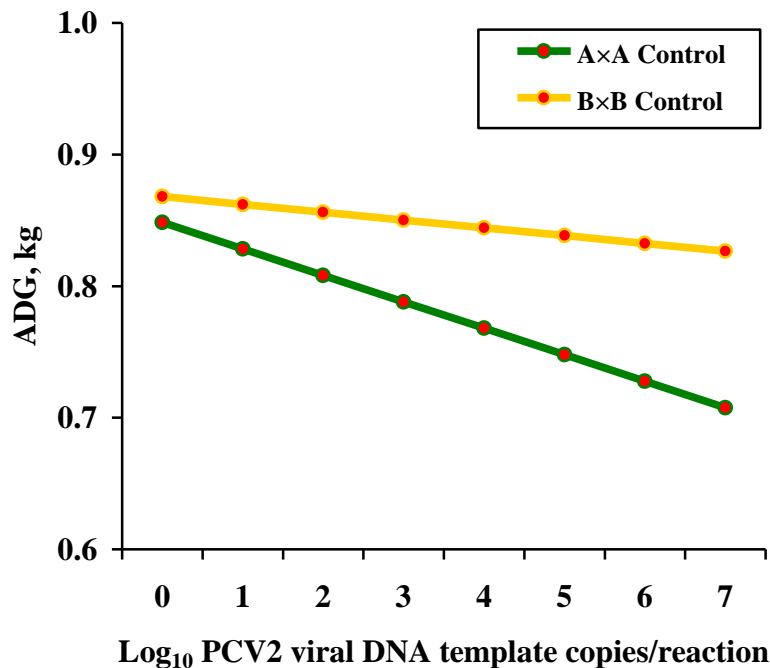


Table 2.1 *P*-values for age, growth performance, and carcass trait response criteria for the interactive effects between genetic makeup and vaccination treatment, and genetic makeup and gender, and the main effects of genetic makeup, vaccination treatment, and gender¹

Item	Genetic ² × Vaccine ³	Genetic × Gender	Genetic	Vaccine	Gender
Age, <i>P</i> <					
D 0	0.71	0.04	0.07	0.48	0.03
D 130	0.41	<0.001	0.11	0.65	<0.001
Weight, <i>P</i> <					
Birth	0.74	0.41	0.25	0.40	0.29
D 0	0.51	0.24	0.32	0.31	0.17
D 130	0.05	0.02	0.003	<0.001	0.86
ADG, <i>P</i> <					
D 0 to 40	0.05	0.22	0.08	0.72	<0.001
D 40 to 130	0.05	0.03	0.003	<0.001	0.20
D 0 to 130	0.04	0.03	0.003	<0.001	0.94
Carcass traits, <i>P</i> <					
Backfat	0.46	0.47	0.02	0.13	0.06
Loin	0.32	0.10	<0.001	<0.001	<0.001
Adj. backfat ⁴	0.79	0.47	<0.01	0.62	0.04
Adj. loin ⁴	0.82	0.01	<0.001	0.29	<0.001

¹ A total of 454 pigs (boar or gilt) from 4 genetic makeups were assigned to vaccination treatment by ranking them by weight within litter and gender and randomly assigning each pig to either a vaccinated or non-vaccinated treatment. Pigs were individually weighed at birth, d 0, 40, and 130. Backfat and loin depth were measured at the time pigs were weighed off test. Analysis was performed by analysis of variance using the GLIMMIX procedure in SAS version 9.1.3 (SAS Institute Inc., Cary, NC) on records from 417 pigs which had complete growth records at off-test. The statistical model included fixed effects of genetic makeup, vaccination treatment, gender, and all interactions. Litter of origin was included as a random effect.

² Genetic designations were A×A (Duroc genotype), A×B, B×A, and B×B (synthetic White Pietrain genotype).

³ Vaccination treatments were vaccinated or non-vaccinated control. Circovirus vaccine (Circumvent PCV; Intervet/Schering-Plough Animal Health, Millsboro, DE) was administered intramuscularly (2 mL per dose) to vaccinated pigs at 21 and 35 d of age.

⁴ Backfat depth and loin depth were adjusted to a common average off-test weight. There was a 3-way interaction (*P* = 0.02) with genetic makeup, vaccination treatment, and gender for weight-adjusted backfat depth. This interaction was a result of control A×B crossbred boars having increased (11.9 ± 0.41 vs. 10.9 ± 0.41 mm, *P* = 0.04) backfat depth compared with vaccinated A×B crossbred boars. Within boars or gilts of A×A, B×A, or B×B, weight-adjusted backfat depth was similar (*P* ≥ 0.09) between control and vaccinated pigs.

Table 2.2 Means and standard errors for age, growth performance, and carcass trait response criteria for control and vaccinated pigs of different genetic makeups¹

Item Treatment ³	Genetic makeup ²							
	A×A		A×B		B×A		B×B	
	Control	Vaccinate	Control	Vaccinate	Control	Vaccinate	Control	Vaccinate
No. of pigs	62	55	60	65	34	32	55	54
Age, d								
D 0	21.2±0.43	21.1±0.43	20.3±0.47	20.3±0.47	21.3±0.65	21.3±0.65	19.7±0.49	19.6±0.49
D 130	151.5±0.46	151.4±0.46	150.6±0.51	150.6±0.51	151.7±0.69	151.7±0.69	150.0±0.52	150.0±0.52
Weight, kg								
Birth	1.6±0.07	1.6±0.08	1.7±0.08	1.8±0.08	1.6±0.11	1.6±0.11	1.8±0.08	1.8±0.08
D 0	5.8±0.23	6.1±0.23	6.3±0.24	6.3±0.24	6.6±0.33	6.5±0.33	5.8±0.25	6.0±0.25
D 130	91.1±2.03 ^a	100.1±2.09 ^b	102.8±2.16 ^{bc}	105.6±2.13 ^{bc}	102.8±2.91 ^{bc}	107.5±2.95 ^c	100.1±2.22 ^b	102.4±2.25 ^{bc}
ADG, g								
D 0 to 40	382±15.7 ^a	388±16.1 ^{abc}	433±16.9 ^{bd}	409±16.7 ^{ace}	437±22.8 ^{bcde}	463±23.0 ^{de}	417±17.4 ^{abcde}	401±17.6 ^{abc}
D 40 to 130	772±17.0 ^a	864±17.6 ^b	873±18.0 ^b	915±17.7 ^c	867±24.2 ^{bc}	909±24.6 ^{bc}	854±18.5 ^b	883±18.8 ^{bc}
D 0 to 130	655±14.7 ^a	721±15.2 ^b	741±15.6 ^{bc}	762±15.4 ^{bc}	738±21.1 ^b	775±21.4 ^c	723±16.1 ^{bc}	739±16.3 ^{bc}
Carcass traits, mm								
Backfat ⁴	11.4±0.34	12.0±0.35	12.1±0.36	12.0±0.35	11.2±0.48	11.7±0.49	10.6±0.37	10.8±0.38
Loin ⁵	59.2±0.87	62.2±0.91	65.6±0.92	66.9±0.90	66.3±1.23	69.0±1.26	68.8±0.95	69.6±0.96
Adj. backfat ⁶	12.2±0.33	12.1±0.33	11.9±0.33	11.6±0.33	11.1±0.45	11.2±0.46	10.7±0.34	10.7±0.35
Adj. loin ⁷	62.3±0.69	62.6±0.69	65.1±0.69	65.4±0.68	65.8±0.92	67.1±0.95	69.2±0.71	69.2±0.72

¹ A total of 454 pigs from 4 genetic makeups were assigned to vaccination treatment by ranking them by weight within litter and gender and randomly assigning each pig to either a vaccinated or non-vaccinated control treatment. Pigs were individually weighed at birth, d 0 (weaning), d 40 (end of nursery period), d 130 (off-test; approximately the end of the finisher period). Backfat and loin depth were measured when pigs were weighed off test. Analysis was performed by analysis of variance using the GLIMMIX procedure in SAS version 9.1.3 (SAS Institute Inc., Cary, NC) on records from 417 pigs which had complete growth records at off-test. The statistical model included fixed effects of genetic makeup, vaccination treatment, gender, and all interactions. Litter of origin was included as a random effect. Results are reported as least squares means \pm SEM.

² Genetic designations were A \times A (Duroc genotype), A \times B, B \times A, and B \times B (synthetic White Pietrain genotype).

³ Vaccination treatments included vaccinated and non-vaccinated controls. A circovirus vaccine (Circumvent PCV; Intervet/Schering-Plough Animal Health, Millsboro, DE) was administered intramuscularly (2 mL per dose) to vaccinated pigs at 21 and 35 d of age.

⁴ Backfat depth was not adjusted to a common weight.

⁵ Loin depth was not adjusted to a common weight.

⁶ Backfat depth was adjusted to a common average off-test weight. There was a 3-way interaction ($P = 0.02$) with genetic makeup, vaccination treatment, and gender for weight-adjusted backfat depth. This interaction was a result of control A \times B crossbred boars having increased (11.9 ± 0.41 vs. 10.9 ± 0.41 mm, $P = 0.04$) backfat depth compared with vaccinated A \times B crossbred boars. Within boars or gilts of A \times A, B \times A, or B \times B, weight-adjusted backfat depth was similar ($P \geq 0.09$) between control and vaccinated pigs.

⁷ Loin depth was adjusted to a common average off-test weight.

^{abcde} Within a row, means without a common superscript letter differ ($P < 0.05$).

CHAPTER 3 - Effects of diet source and vaccination for porcine circovirus type 2 and *Mycoplasma hyopneumoniae* on nursery pig performance

Abstract

Two experiments were conducted to evaluate the effects of segregated early weaning (SEW) and transition diet sources, circovirus and *Mycoplasma hyopneumoniae* (*M. hyo*) vaccinations, and vaccination timing on pig performance. In experiment one, a total of 400 pigs (5.6 ± 1.03 kg BW) were used in a 20-d study. Treatments were arranged in a 4×2 factorial in a randomized complete block design (5 pigs per pen and 10 pens per treatment) with main effects of diet manufacturing source (A, B, C, or D) and vaccination timing (d 0 or 8). Vaccines (Circumvent PCV; Intervet/Schering-Plough Animal Health, Millsboro, DE, and RespiSure One; Pfizer Animal Health, New York, NY) were administered on d 0 (weaning) or d 8. A budgeted amount of SEW diet (0.45 kg/pig) was fed followed by a transition diet until d 8 and a common diet from d 8 to 20. Diet source affected ($P < 0.001$) ADG during the first 4 d and affected ($P \leq 0.02$) ADG and ADFI from d 4 to 8. From d 0 to 4, pigs fed diet B had increased ($P \leq 0.03$) ADG compared with pigs fed diets A, C, or D. Growth rate of pigs fed diet D was increased ($P = 0.02$) compared with pigs fed diet C and ADG of pigs fed diet A was intermediate. From d 4 to 8, pigs fed diet A had increased ($P = 0.04$) ADG compared with pigs fed diet B, and had increased ($P \leq 0.04$) ADG and ADFI compared with pigs fed diets C or D. Pigs fed diet B had increased ($P \leq 0.02$) ADFI compared with pigs fed diet C or D. There were no differences ($P \geq 0.18$) among diet sources once pigs were fed a common diet (d 8 to 20). Overall, diet source did not affect ADG; but ADFI tended ($P = 0.06$) to be decreased for pigs fed diet C compared with those fed diets A, B, and D. Pigs vaccinated on d 0 had decreased ($P \leq 0.01$) ADG and ADFI (d 4 to 8 and d 0 to 8), resulting in lighter ($P = 0.003$) weights on d 8 than those of pigs not yet vaccinated (d 8). However, overall ADG was not affected by vaccination timing. In experiment two, 360 pigs (5.9 ± 0.91 kg BW) were used in a 35-d trial to evaluate the effects of vaccination for circovirus and *M. hyo* using different vaccines. Treatments were arranged in a 3×2 factorial in a randomized complete block design (5 pigs per pen and 12 pens per treatment). Main effects

included circovirus vaccine (none; CircoFLEX, Boehringer Ingelheim Vetmedica, Inc, St. Joseph, MO; or Circumvent PCV); with or without a *M. hyo* vaccine (RespiSure, Pfizer Animal Health, New York, NY).

Overall, pigs vaccinated with Circumvent PCV had decreased ($P < 0.02$) ADG and ADFI compared with pigs vaccinated with CircoFLEX or controls. On d 35, pigs vaccinated with Circumvent PCV weighed less ($P < 0.01$) than CircoFLEX-vaccinated or control pigs. RespiSure-vaccinated pigs had decreased ($P \leq 0.05$) ADG compared with control pigs from d 14 to 21 and d 21 to 29. On d 35, RespiSure-vaccinated pigs tended ($P = 0.06$) to weigh and consume less than control pigs. These data indicate diet source and vaccination timing affects pig performance after weaning. Vaccination for PCV2 and *M. hyo* independently reduced ADG and ADFI, but the effect was product-dependent. These factors should be considered when managing pigs after weaning.

Key words: diet, growth, *M. hyo*, nursery pig, PCV2, vaccine

Introduction

Growth performance of pigs immediately after weaning affects performance throughout the growing and finishing phases of production (Tokach et al., 1992). In addition, increased feed intake during the first wk after weaning reduces the risk of development of digestive disorders (Madec et al., 1998). Although weaning is a stressful event known to affect performance, in late 2007, reports from producers and veterinarians in the field indicated increased difficulty starting or maintaining pigs on feed immediately after weaning. The increase in reports seemed to coincide with the widespread adoption of vaccination for porcine circovirus type 2 (PCV2) possibly indicating that circovirus vaccination may be contributing to the producer-noted difficulty in starting pigs on feed. These vaccines are labeled for administration at a similar time when other growing pig vaccines, such as *Mycoplasma hyopneumoniae* (*M. hyo*) vaccines, are administered. However, throughout the United States swine industry, timing of circovirus vaccination has not been consistent. In addition, other management factors are known to affect nursery pig performance, such as diet formulation (Dritz et al., 1996) and weaning age (Main et al., 2004). However, there are limited data on the effects of diet manufacturing source on postweaning performance. Therefore, the objectives of this series of two experiments were to:

(1) investigate the effects of diet manufacturing source and vaccination timing for circovirus and *M. hyo* vaccines on nursery pig performance, and (2) determine the effects of 2 commercial circovirus vaccines and a *M. hyo* vaccine on nursery pig performance.

Materials and Methods

Procedures used in these experiments were approved by the Kansas State University Institutional Animal Care and Use Committee. Both experiments were performed at the Kansas State University Segregated Early Wean facility. All pens (1.2 m × 1.2 m) were equipped with a single cup waterer and a 4-hole, dry, self-feeder providing pigs with ad libitum access to water and feed.

In experiment one, a total of 400 weanling pigs (PIC 1050, PIC, Hendersonville, TN) were used in a 20-d growth study to evaluate the effects of segregated early weaning (SEW) and transition diet source and timing for circovirus and *M. hyo* vaccination on nursery pig performance. Pigs (5.6 ± 1.03 kg BW) were blocked by weight and assigned to pens (5 pigs per pen) which resulted in 10 blocks of 8 pens with pigs of comparable weight and gender characteristics. A total of 31 gilts and 369 barrows were included in this trial. Gilts were distributed among pens within a single block with 7 pens accommodating 4 gilts and 1 pen having 3 gilts. Treatments were arranged in a 4 × 2 factorial in a randomized complete block design (10 pens per treatment) with main effects of diet manufacturing source (A, B, C, or D) and vaccination timing (d 0 or d 8). Within a block, each pen was randomly assigned to 1 of the 8 treatment combinations. All SEW and transition diets were formulated to similar nutrient specifications (Table 3.1) and were obtained from 4 commercial feed manufacturers.

At weaning (d 0), a total of 0.45 kg SEW diet was budgeted per pig. For pens within a diet source, after the SEW diet was placed in the feeders, a transition diet was added on top of the SEW diet and was fed to pigs until d 8. On d 8, feeders were emptied prior to feeding a common Phase 2 diet until d 20.

Single doses of circovirus and *M. hyo* vaccine were administered to pigs during the 20-d trial with all pigs receiving a second dose of circovirus vaccine after being weighed off test. According to vaccination timing treatment assignment, pigs were vaccinated with 2 mL Circumvent PCV (Intervet/Schering-Plough Animal Health, Millsboro, DE) and 2 mL RespiSure One (Pfizer Animal Health, New York, NY) on either d 0 or d 8. All vaccines were administered

as separate intramuscular injections in the neck according to vaccine label instructions. Pigs were weighed and feed disappearance was determined on d 0, 4, 8, and 20. From these data, pen-level ADG, ADFI, and G:F were calculated. If a pig was removed from a pen, the date of removal and pig weight were recorded for use in performance calculations.

In experiment two, a total of 360 weanling barrows (PIC 1050; 5.9 ± 0.91 kg BW) were used in a 35-d growth trial to evaluate the effects of vaccination for circovirus and *M. hyo* on nursery pig performance. At weaning (d 0), barrows were blocked by body weight and assigned to pens (5 pigs per pen) to create 12 blocks of 6 pens with each pen housing similar weight pigs. Within blocks, pens of pigs were randomly allotted to 1 of 6 treatments in a 3×2 factorial arrangement. Main effects included circovirus vaccine and *M. hyo* vaccine. The circovirus vaccine treatments were: (1) no circovirus vaccine (non-circovirus-vaccinated control); (2) a 2-dose vaccine, Circumvent PCV; and (3) a 1-dose vaccine, Ingelvac CircoFLEX (Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO). The *M. hyo* vaccine treatments were: (1) no *M. hyo* vaccine (non-*M. hyo*-vaccinated control); and (2) a 2-dose vaccine, RespiSure (Pfizer Animal Health, New York, NY). All vaccines were administered as separate intramuscular injections according to label directions (Circumvent PCV: 2 mL per dose given on d 0 and 21; CircoFLEX: 1 mL per dose given on d 0; RespiSure: 2 mL per dose given on d 0 and 21).

All pigs were phase fed a similar diet within phase throughout the trial. Initially, 0.45 kg SEW diet was budgeted per pig, followed a transition diet until d 8. All pigs were then fed a common Phase 2 diet from d 8 to 21 and a common Phase 3 diet from d 21 to 35. Feeders were emptied on d 8 and d 21 prior to switching to Phase 2 and 3 diets, respectively. Pigs were weighed and feed disappearance was determined on d 0, 4, 8, 14, 21, 25, 29, and 35. From these data, pen-level ADG, ADFI, and G:F were calculated. If a pig was removed from a pen, the date of removal and pig weight were recorded for use in performance calculations.

Data for both experiments were analyzed as a randomized complete block designs using the GLIMMIX procedure in SAS version 9.1.3 (SAS Institute Inc., Cary, NC) with pen as the experimental unit. For experiment one, fixed effects were diet source, vaccination timing, and their interaction. For experiment two, fixed effects included circovirus vaccine, *M. hyo* vaccine, and their interaction. Weaning weight, the blocking factor, was included as a random effect for both experiments. Differences between treatments were determined using least squares means ($P < 0.05$).

Results

Experiment one:

For experiment one, there were no 2-way interactions between diet source and vaccination timing for any response criteria with the exception of d 0 to 8 G:F. The interaction ($P = 0.05$; Table 3.2) resulted from a crossing interaction with pigs fed diets from source B and not yet vaccinated having poorer ($P = 0.04$) G:F compared with vaccinated pigs fed diets from source B. Within all other diet source treatments, pigs not yet vaccinated (d 8) had numerically increased or similar ($P \geq 0.07$) G:F as pigs vaccinated on d 0.

For the remainder of the responses, the main effects of diet source (Table 3.3) and vaccine timing (Table 3.4) are reported. From d 0 to 4, approximately the time the budgeted amount of SEW diet was consumed, diet source affected ($P < 0.001$) ADG but not ADFI ($P = 0.22$) or G:F ($P = 0.28$). Pigs fed diets from source B had increased ($P \leq 0.03$) ADG and d 4 weights compared with pigs fed diets from sources A, C, or D. Pigs fed diets from source D grew faster ($P = 0.02$) compared with pigs fed diet C and pigs fed diet A had intermediate ADG.

From d 4 to 8, pigs fed diets from source A had increased ($P \leq 0.04$) ADG compared with pigs fed diets from sources B, C, or D. Contributing to this greater ADG was an increase ($P \leq 0.04$) in feed consumption for pigs fed diet A compared with pigs fed diets from sources C or D. In addition, pigs fed diets from source B had improved ($P \leq 0.02$) ADFI compared with pigs fed diets from sources C or D. Diet source tended ($P = 0.07$) to affect G:F with pigs fed diets from source A having improved G:F than that of pigs fed diets from sources B, C, or D.

From d 0 to 8, diet source affected ($P \leq 0.001$) rate of gain and feed consumption. Pigs fed diets from source C had lower ($P \leq 0.01$) ADG and ADFI compared with pigs fed diets from sources A or B while pigs fed diets from source D had intermediate ADG and ADFI. These performance differences resulted in pigs fed SEW and transition diets from sources A and B being 0.3 kg heavier ($P < 0.001$) on d 8 compared with pigs fed similarly-formulated diets from source C.

After pigs were placed on a common diet (d 8), diet source did not continue to affect (d 8 to 20: $P \geq 0.18$) pig performance. Overall (d 0 to 20) ADG and G:F were not affected (ADG: $P \geq 0.26$) by diet source; however, a trend ($P = 0.06$) was observed for an effect of diet source on ADFI. This suggested that pigs fed diets from source C had lower intake compared with pigs fed diets from sources A, B, or D.

There was no immediate effect ($P \geq 0.20$) of vaccination on pig performance from d 0 to 4 for pigs vaccinated on d 0 compared with pigs not yet vaccinated. From d 4 to 8, pigs vaccinated with RespiSure One and Circumvent PCV had decreased ($P \leq 0.01$) ADG and ADFI compared with pigs not yet vaccinated. Therefore, during the first 8 d after vaccination, pigs vaccinated on d 0 had reduced ($P \leq 0.01$) ADG and ADFI that resulted in vaccinated pigs weighing 0.2 kg less ($P = 0.003$) on d 8 compared with pigs not yet vaccinated.

From d 8 to 20, pigs vaccinated on d 8 had decreased ($P = 0.05$) ADG compared with pigs vaccinated on d 0. There was no effect ($P \geq 0.12$) of vaccination timing on ADFI or G:F for this period.

Overall, after all pigs had been vaccinated, vaccination timing did not affect ($P \geq 0.40$) ADG, ADFI, or G:F. Pigs had similar ($P = 0.78$) d 20 weights whether they were vaccinated with RespiSure One and Circumvent PCV at weaning or 8-d after weaning.

Experiment two:

In experiment two, there were no 2-way interactive effects between circovirus vaccine and *M. hyo* vaccine ($P \geq 0.06$) observed for any response criteria, hence main effects of circovirus vaccine (Table 3.5) and *M. hyo* vaccine (Table 3.6) are reported. Within the first 8 d after vaccination (d 0 to 8), circovirus vaccine treatment did not affect ($P \geq 0.26$) ADG or G:F. However, pigs vaccinated with Circumvent PCV had decreased ($P = 0.01$) ADFI compared with pigs vaccinated with CircoFLEX and intake of non-circovirus-vaccinated pigs was intermediate. From d 8 to 14, pigs vaccinated with Circumvent PCV had reduced ($P < 0.03$) feed intake compared with pigs vaccinated with CircoFLEX and non-circovirus-vaccinated pigs. There was no effect ($P \geq 0.12$) of circovirus vaccine treatment on ADG or ADFI from d 14 to 21; however, G:F was improved ($P = 0.02$) for pigs vaccinated with Circumvent PCV compared with pigs vaccinated with CircoFLEX and non-circovirus-vaccinated pigs had intermediate G:F.

In the 8-d following the second vaccination for the Circumvent PCV treatment (d 21 to 29), pigs vaccinated with Circumvent PCV had decreased ($P < 0.01$) ADG and ADFI compared with both CircoFLEX-vaccinated pigs and non-circovirus-vaccinated pigs. Pigs vaccinated with Circumvent PCV tended to have poorer ($P = 0.08$) G:F compared with pigs vaccinated with CircoFLEX or non-circovirus-vaccinated pigs. From d 29 to 35, pigs vaccinated with Circumvent PCV tended to have reduced ($P = 0.10$) ADFI compared with CircoFLEX-

vaccinated and non-circovirus-vaccinated pigs; however, ADG and G:F were not affected ($P \geq 0.13$) by circovirus vaccine treatment.

Overall (d 0 to 35), rate of gain and feed intake were both reduced ($P < 0.02$) in pigs vaccinated with Circumvent PCV compared with pigs either vaccinated with CircoFLEX or those not vaccinated for circovirus. Growth rate and feed consumption were similar ($P \geq 0.34$) between CircoFLEX-vaccinated pigs and non-circovirus-vaccinated pigs. Overall G:F was not affected ($P = 0.30$) by circovirus vaccine treatment. The reduced ADFI and ADG for pigs vaccinated with Circumvent PCV resulted in pigs vaccinated with Circumvent PCV weighing less ($P < 0.01$) on d 35 compared with CircoFLEX-vaccinated pigs or non-circovirus-vaccinated pigs.

The *M. hyo* vaccine treatment results indicate that nursery pig performance was not affected ($P \geq 0.40$) by vaccination in the first 8-d following initial injection. From d 14 to 21, pigs vaccinated with RespiSure tended to have reduced ($P = 0.05$) ADG compared with non-*M. hyo*-vaccinated pigs. There was no difference ($P \geq 0.18$) between *M. hyo* vaccine treatments for ADFI or G:F from d 14 to 21.

Following the second injection of RespiSure (d 21), pigs had reduced ($P \leq 0.02$) ADG and ADFI from d 21 to 29. During this period, G:F was not affected ($P = 0.85$) by *M. hyo* vaccine treatment. Feed intake was affected by vaccine treatment from d 29 to 35 with pigs vaccinated with RespiSure consuming less ($P = 0.03$) feed than non-*M. hyo*-vaccinated pigs. Overall (d 0 to 35), ADG and feed intake tended ($P \leq 0.10$) to be reduced for pigs vaccinated with RespiSure compared with non-*M. hyo*-vaccinated pigs. These performance differences resulted in pigs vaccinated with RespiSure tending ($P = 0.06$) to weigh less on d 35 compared with non-*M. hyo*-vaccinated control pigs.

Although there was no 2-way interaction between circovirus vaccine treatments and *M. hyo* vaccine treatments ($P = 0.68$) observed, analysis of d-35 weights for the circovirus \times *M. hyo* vaccine treatments measured against non-vaccinated control pigs demonstrated that approximately a 0.7 kg reduction in weight may be due to Circumvent PCV vaccination and an additional 0.5 kg reduction in weight may be due to RespiSure vaccination. Therefore, when Circumvent PCV and RespiSure vaccines were administered in conjunction, the negative effects were additive and resulted in a 1.2 kg lighter mean weight on d 35 (Figure 3.1) compared with the mean weight of the non-vaccinated control pigs.

Discussion

Immediately after weaning, nursery pigs are exposed to a variety of stressors such as dietary changes, adaptation to a new environment, and alteration in social structure. In addition, vaccines against several pathogens, including PCV2 and *M. hyo*, are often administered at a similar time. It has been reported that stress effects on growth performance are additive; thus, growth can be affected by removal of non-essential stressors (Hyun et al., 1998).

Some stressful events are unavoidable as pigs must be weaned from sows, where they consumed a milk-only diet, and moved to locations where they are often offered a pelleted diet. It is known that diet formulation impacts nursery pig performance; however, there are limited reports that describe how variability in diets resulting from factors other than formulation could affect pig performance.

The results from experiment one indicate that commercial source of SEW and transition diet should be considered when selecting nursery pigs diets. Diet sources A, B, and C manufactured SEW diets formulated to identical nutrient and ingredient specifications. Growth performance of pigs fed diets from source B for the first 4 d after weaning was improved compared to that of pigs fed diets from the other sources. These findings of variable growth performance of pigs fed similarly formulated SEW diets suggest that factors other than formulation must be evaluated and considered when selecting nursery pigs diets and ingredients. For example, use of different lactose sources in diets which are similarly formulated could affect pig performance because of product quality differences between the sources (Nessmith et al., 1997).

Manufacturing process can affect ingredient and pellet quality which in turn can impact the quality of the diet. Variability between ingredient sources has been demonstrated for products such as whey protein concentrate (Gottlob et al., 2005). In our study, the reason for the variability between sources was not determined. It is also not known whether the characteristics responsible for the between-source differences would remain consistent over time. These diet source effects may be dynamic as mills change suppliers for ingredients or buy ingredients over time. Our experiment was designed only to determine at a single time point whether there were differences between commercial milling sources for similarly-formulated SEW and transition diets which would affect nursery pig performance.

Another factor that affected pig performance immediately after weaning was vaccination, though the effect appeared to be product-dependent. In both experiments one and two, vaccination of weanling pigs with Circumvent PCV and either RespiSure One or RespiSure negatively impacted pig performance after vaccination. By d 8 in experiment one, pigs vaccinated with both Circumvent and RespiSure One had decreased ADG and weighed 0.2 kg less on d 8 than pigs not yet vaccinated. In contrast, in experiment two, the numeric differences in d 8 weights of non-vaccinates (6.9 ± 0.30 kg BW) compared to pigs vaccinated with Circumvent PCV and RespiSure (6.8 ± 0.30 kg BW, data not shown), indicated there was a 0.1 kg weight difference by d 8. Therefore, the magnitude of the growth responses within the first 8 days after weaning and first injection of Circumvent PCV with an additional *M. hyo* vaccine product was greater in experiment one than in experiment two.

The differences may have resulted from the different *M. hyo* vaccines used between the experiments. Additionally, some of the inconsistency may have been due to pigs in experiment two experiencing several challenges around the time of weaning. These challenges consisted of heat stress experienced during trucking followed by several pigs exhibiting lameness associated with a bacterial infection. Also, in experiment two, pigs were fed common SEW and transition diets which were purchased from the same source as diet source C in experiment one. Pigs fed the SEW and transition diets from source C did not consume those diets well in our first experiment. All of these factors could have contributed to variability in growth rates after weaning in experiment two.

Overall results from experiment one indicate that growth performance and feed intake were not different between the vaccination timing treatments. Thus, vaccination appeared to cause a reduction in growth rate of pigs whether they were vaccinated with Circumvent PCV and RespiSure One at weaning or 8 d later. As there was no non-vaccinated control after d 8, the severity of the vaccination effect between the timing treatments was not clearly defined. It was also not possible to determine whether vaccinated pigs compensated in subsequent growth rate to overcome the negative effects of vaccination on growth performance.

The experimental design of experiment two allowed the effects of individual vaccine products on performance to be determined. Vaccination with Circumvent PCV alone contributed to reduction in feed intake immediately after vaccination. Interestingly, the majority of the negative effect occurred after the second vaccination. In contrast, vaccination with CircoFLEX,

a 1-dose circovirus vaccine, did not affect performance compared with non-circovirus-vaccinated pigs.

By d 35, pigs vaccinated with Circumvent PCV had not compensated with increased growth rate to return to a similar weight as the non-circovirus-vaccinated pigs or the CircoFLEX-vaccinated pigs. Results from a different field study support these findings as it was determined that pigs vaccinated with a 2-dose circovirus vaccine grew slower during the nursery period than pigs vaccinated with a 1-dose circovirus vaccine or non-vaccinated control pigs (Vilaca et al., 2010).

An immediate negative effect following circovirus vaccination with the 2-dose circovirus vaccine has been observed in other studies though the effect has either not been consistent or was not maintained. In one study, circovirus vaccine administered at 9 and 11 wk of age resulted in vaccinated pigs being 0.8 kg lighter ($P < 0.02$) than non-vaccinated controls just 15 d after administration of the first dose and 1 d after the second dose of circovirus vaccine (Jacela et al., 2011). In a different study, compared with non-circovirus-vaccinated pigs under commercial conditions, Shelton et al. (2009) observed a reduction ($P < 0.04$) in growth rate and feed intake in a 2 wk period following administration of the second dose of circovirus vaccine (Shelton et al., 2009). Other studies evaluating the 2-dose circovirus vaccine have failed to detect any differences in nursery pig growth rate following circovirus vaccination (Horlen et al., 2008). While the effects following vaccination have been variable, the results of some studies have indicated that, after pigs are exposed to conditions with field-virus challenge, circovirus vaccination was associated with improved growth rates (Horlen et al., 2008; Jacela et al., 2011).

The effects of the 2-dose *M. hyo* vaccine (RespiSure) on performance in experiment two mirror the effect patterns of the 2-dose circovirus vaccine. Overall, pigs vaccinated with RespiSure tended to weigh less on d 35 with the majority of the negative impacts on feed intake and growth rate occurring after administration of the second dose of vaccine.

The results of experiment two indicate that the effects of the circovirus and *M. hyo* vaccine used were not antagonistic or multiplicative, but were additive. This is consistent with previous research which determined that stress effects were additive in nature (Hyun et al., 1998). Thus, the benefit that could be achieved by the removal of a single stressor should be predictable. In experiment two, approximately a 0.7 kg reduction in weight on d 35 was due to Circumvent PCV vaccine alone and an additional 0.5 kg reduction in weight was due to

RespiSure vaccination (Figure 3.1). There was no negative effect on d 35 weight associated with CircoFLEX vaccination. Therefore, the effects on nursery pig performance appeared to be vaccine product-dependent.

Reasons for the performance-altering differences among products at this time are not well defined. However, these products vary with respect to adjuvant as well as antigen composition. Adjuvants affect growth performance and reproduction rate as well as impact animal comfort or carcass quality (Aucouturier et al., 2001). RespiSure One and RespiSure are manufactured using Amphigen, a proprietary oil adjuvant with surfactant (Hoogland et al., 2006; Kuhn, 2004), creating an oil-in-water emulsion vaccine.

Circumvent PCV is made with Microsol Diluvac Forte (Thacker, 2006), a proprietary dual oily emulsion adjuvant. Ingelvac CircoFLEX is manufactured using ImpranFLEX (Roof, 2010), a proprietary aqueous-based polymer adjuvant. Although adjuvants without oil may be more easily tolerated, the oil phase creates a depot for longer release of antigen leading to potential differences in development of or duration of immunity against disease (Aucouturier et al., 2001). Water-in-oil emulsions reportedly induced higher levels of antibody (Jansen et al., 2006), yet these emulsions have the potential to be damaging to tissues due to the high oil content and recruitment of inflammatory cells (Aucouturier et al., 2001). Although beneficial for increased antigen presentation and antibody production, the tissue damage has potential to produce a more pronounced inflammatory response that reduces feed intake and growth rate.

Differences in immune system stimulation by different antigens could contribute to differences in performance due to the physiologic cost to development of immunity. Derting and Compton (2003) reported that in white-footed mice more energy was required to produce an immune response compared with maintenance of immunity. They suggested that there were 2 mechanisms from which energy was obtained: (1) allocating it away from other physiologic processes, or (2) increasing feed intake. Their results indicated that energy was directed away from other physiologic systems instead of promoting increased feed intake for the mouse (Derting and Compton, 2003). It is possible that immune system activation caused redirection of nutrients from productive growth to development of protective immunity or immune response necessary to counteract disease processes (Colditz, 2002). It was reported that during the height of an immune response, metabolic profiles of cells shifted and oxygen consumption and glucose utilization increased by two-fold per cell (Colditz, 2002). Therefore, there was a metabolic cost

to development of immunity. In pigs, Schinckel et al. (1995) determined that immune system activation by antigens including numerous vaccines and *Escherichia coli* lipopolysaccharide administered to early-weaned nursery pigs negatively affected growth rate and feed intake compared with control pigs not exposed to these same antigens during the nursery period. Under university research conditions, the antigen-exposed pigs took approximately 4 days longer than control pigs to reach 120 kg (Schinckel et al., 1995).

Despite the negative effects associated with administration of some nursery pig vaccines, it has been documented that circovirus vaccines and *M. hyo* vaccines improve pig performance or reduce lesion severity under field conditions with notable disease challenge (Dohoo and Montgomery, 1996; Horlen et al., 2008; Scheidt et al., 1994). Therefore, results from these experiments should not be interpreted as advocating removal of these vaccines from disease-affected production systems. However, the findings in our experiments suggest potential negative effects of vaccine usage should be considered when implementing nursery pig vaccination strategies. Altering vaccination timing may delay these effects and may be a strategy used if pigs are experiencing multiple stressors within a short period; however, timing of vaccination is sometimes dictated by the length of time before natural exposure to the pathogen occurs. Vaccination timing and product factors should be considered when developing circovirus and *M. hyo* vaccination protocols to achieve optimum vaccine efficacy and minimize negative effects on the nursery pig.

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Figure 3.1 The effect of circovirus vaccination and *Mycoplasma hyopneumoniae* (*M. hyo*) vaccination on d-35 pig weight (Exp. 2).

A total of 360 weanling barrows (PIC 1050; 5.9 ± 0.91 kg BW) were used in a 35-d trial to evaluate the effects of vaccination for circovirus and *M. hyo* on pig performance. At weaning (d 0), barrows were ranked by body weight and assigned to pens (5 pigs per pen). Within blocks, pens of pigs were randomly allotted treatments in a 3×2 factorial arrangement with main effects of circovirus vaccine and *M. hyo* vaccine. Circovirus vaccine treatments were: (1) Circovirus controls: No circovirus vaccine; (2) Circumvent PCV: pigs vaccinated with 2 mL Circumvent PCV (Intervet/Schering-Plough Animal Health, Millsboro, DE) administered intramuscularly on d 0 and 21; and (3) CircoFLEX: pigs vaccinated with 1 mL Ingelvac CircoFLEX (Boehringer Ingelheim Vetmedica, Inc, St. Joseph, MO) administered intramuscularly on d 0. *M. hyo* vaccine treatments were: (1) *M. hyo* controls: No *M. hyo* vaccine; and (2) RespiSure (Pfizer Animal Health, New York, NY) pigs vaccinated with 2 mL RespiSure administered intramuscularly on d 0 and 21. Data were analyzed using the GLIMMIX procedure of SAS version 9.1.3 (SAS Institute Inc., Cary, NC) with pen as the experimental unit. The model included the fixed effects of circovirus vaccine, *M. hyo* vaccine, and their interaction, and a random effect of weight block.

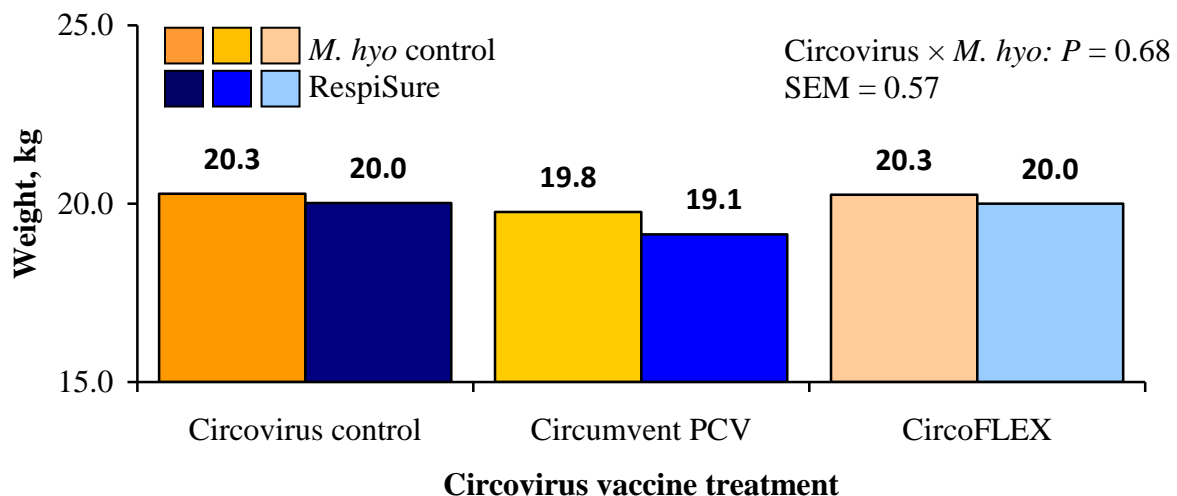


Table 3.1 Composition of segregated early wean (SEW) and transition diets (Exp. 1)

Ingredient, %	Diet type				
	SEW (sources A, B, and C)	SEW (source D) ¹	Transition ² (sources A, B, and C)	Transition ² (sources A, B, and C)	Transition (source D) ¹
Corn	33.70	25.60	37.70	37.25	26.35
Soybean meal (46.5% CP)	12.55	12.70	20.00	20.00	21.55
Spray-dried porcine plasma	6.70	6.70	2.50	2.50	2.50
Select menhaden fish meal	6.00	6.00	5.80	5.00	6.00
Spray-dried blood cells	1.65	1.65	1.25	1.25	1.25
Spray-dried whey	25.00	--	12.50	25.00	--
Whey permeate	6.00	25.00	11.25	--	20.00
Ground oat groats	--	15.00	--	--	15.00
Choice white grease	5.00	3.00	5.00	5.00	3.00
Monocalcium phosphate (21% P)	0.30	0.50	0.60	0.70	0.60
Limestone	0.45	0.60	0.45	0.45	0.60
Salt	0.25	0.25	0.30	0.30	0.30
Zinc oxide	0.36	0.36	0.36	0.36	0.36
Vitamin premix with phytase	0.25	0.25	0.25	0.25	0.25
Trace mineral premix	0.15	0.15	0.15	0.15	0.15
L-lysine HCl	0.15	0.30	0.30	0.26	0.30
DL-methionine	0.15	0.23	0.20	0.18	0.19
L-threonine	0.08	0.14	0.15	0.13	0.16
L-isoleucine	--	0.15	--	--	0.05
Antibiotic ³	1.00	1.00	1.00	1.00	1.00
Acidifier	0.20	0.35	0.20	0.20	0.35
Vitamin E, 20,000 IU	0.05	0.05	0.05	0.05	0.05
Total	100.00	100.00	100.00	100.00	100.00

Calculated analysis

Standard ileal digestible amino acids

Lysine, %	1.57	1.57	1.50	1.51	1.50
Methionine:lysine, %	30	34	35	33	35
Met & Cys:lysine, %	55	57	56	55	56
Threonine:lysine, %	64	62	62	63	62
Tryptophan:lysine, %	17	18	17	17	18
Total lysine, %	1.71	1.69	1.63	1.65	1.63
ME, kcal/kg	3,499	3,430	3,490	3,472	3,413
Protein, %	22.80	22.50	21.80	22.20	22.70
Calcium, %	0.82	0.84	0.82	0.83	0.85
Phosphorus, %	0.76	0.79	0.75	0.77	0.77
Available phosphorus, %	0.59	0.58	0.54	0.55	0.52

¹ Source D SEW and transition diets were formulated differently from diets supplied by sources A, B, and C due to higher costs of whey at the time of formulation.

² Diet sources A, B, and C supplied identically formulated SEW diets, but had the option of using either permeate or whey in their transition diets.

³ Antibiotics included were tiamulin (35 g/ton) and chlortetracycline (400 g/ton).

Table 3.2 Means for the effects of diet source and vaccination timing on nursery pig performance¹ (Exp. 1)

Diet source:	A		B		C		D		Source × Timing	
Item Timing, d:	0	8	0	8	0	8	0	8	SEM	<i>P</i> <
d 0 to 4										
ADG, g	171	188	217	219	162	162	180	203	13.6	0.67
ADFI, g	107	111	119	133	103	101	137	118	14.8	0.71
G:F	1.61	1.74	1.83	1.65	1.60	1.57	1.67	1.73	0.084	0.29
d 4 to 8										
ADG, g	232	257	212	220	174	211	176	222	16.0	0.54
ADFI, g	249	256	245	272	202	226	171	249	20.1	0.25
G:F	0.94	1.01	0.88	0.81	0.85	0.93	0.60	0.89	0.085	0.22
d 0 to 8										
ADG, g	202	222	215	219	168	187	178	213	11.0	0.43
ADFI, g	178	183	182	203	152	164	154	183	10.2	0.51
G:F	1.14 ^{abc}	1.23 ^a	1.19 ^{ab}	1.09 ^c	1.10 ^{bc}	1.14 ^{abc}	1.16 ^{abc}	1.16 ^{abc}	0.035	0.05
d 8 to 20										
ADG, g	311	308	313	305	333	294	325	320	13.1	0.25
ADFI, g	422	429	424	417	422	399	429	430	17.4	0.50
G:F	0.74	0.72	0.74	0.73	0.79	0.74	0.76	0.75	0.022	0.45
d 0 to 20										
ADG, g	267	274	274	271	267	250	266	277	10.4	0.30
ADFI, g	324	330	327	331	314	304	319	331	13.1	0.57
G:F	0.83	0.83	0.84	0.82	0.85	0.82	0.84	0.84	0.017	0.61
Weight, kg										
d 0	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	0.27	0.82
d 4	6.4	6.4	6.6	6.6	6.3	6.4	6.4	6.5	0.30	0.87
d 8	7.3	7.5	7.4	7.4	7.0	7.2	7.1	7.4	0.33	0.52
d 20	11.0	11.2	11.0	11.1	10.9	10.8	11.0	11.1	0.44	0.79

¹A total of 400 weanling pigs were used in a 20-d growth trial to evaluate the effects of segregated early wean and transition diet source (A, B, C, and D) and vaccination timing (d 0 or d 8). There were 5 pigs per pen and 10 pens per diet source and vaccination timing treatment combination. All SEW and transition diets were formulated to similar nutrient specifications but were obtained from 4 commercial sources. A single dose of circovirus and *Mycoplasma hyopneumoniae* vaccine was administered to pigs during 20-d trial with a second dose of circovirus vaccine given after pigs were weighed off test. According to vaccination timing treatment assignment, pigs were vaccinated with 2 mL Circumvent PCV (Intervet/Schering-Plough Animal Health, Millsboro, DE) and 2 mL RespiSure One (Pfizer Animal Health, New York, NY) on either d 0 or 8. Data were analyzed using the GLIMMIX procedure of SAS version 9.1.3 (SAS Institute Inc., Cary, NC) with pen as the experimental unit. Fixed effects used in the model were diet source, vaccination timing, and their interaction. Weight block was included as a random effect.

^{abc} Within a row, means without a common superscript letter differ ($P < 0.05$).

Table 3.3 Means for the effect of diet source on nursery pig performance¹ (Exp. 1)

Item	Diet source				SEM	Probability, <i>P</i> <
	A	B	C	D		
d 0 to 4						
ADG, g	180 ^{ab}	218 ^c	162 ^a	191 ^b	10.9	< 0.001
ADFI, g	109	126	102	127	10.7	0.22
G:F	1.67	1.74	1.59	1.70	0.061	0.28
d 4 to 8						
ADG, g	244 ^a	216 ^b	192 ^b	199 ^b	12.7	0.002
ADFI, g	252 ^a	259 ^a	214 ^b	210 ^b	15.3	0.02
G:F	0.97	0.84	0.89	0.75	0.060	0.07
d 0 to 8						
ADG, g	212 ^{ab}	217 ^a	177 ^c	195 ^{bc}	9.0	< 0.001
ADFI, g	181 ^{ab}	192 ^a	158 ^c	169 ^{bc}	8.2	0.001
G:F	1.18	1.14	1.12	1.16	0.026	0.27
d 8 to 20						
ADG, g	309	309	314	323	10.9	0.52
ADFI, g	425	421	410	429	15.8	0.29
G:F	0.73	0.74	0.77	0.76	0.017	0.18
d 0 to 20						
ADG, g	270	272	259	272	8.8	0.26
ADFI, g	327	329	309	325	11.8	0.06
G:F	0.83	0.83	0.84	0.84	0.013	0.80
Weight, kg						
d 0	5.7	5.7	5.7	5.7	0.27	0.80
d 4	6.4 ^a	6.6 ^b	6.3 ^a	6.4 ^a	0.30	< 0.001
d 8	7.4 ^{ab}	7.4 ^a	7.1 ^c	7.2 ^{bc}	0.32	< 0.001
d 20	11.1	11.1	10.8	11.0	0.43	0.35

¹A total of 400 weanling pigs were used in a 20-d growth trial to evaluate the effects of segregated early wean and transition diet source (A, B, C, and D) and vaccination timing (d 0 or 8). There were 5 pigs per pen and 20 pens per diet source treatment. Four commercial diet sources produced similarly formulated SEW and transition pelleted diets for this experiment. Initially, 0.45 kg SEW diet was budgeted per pig after which transition diets were fed until d 8 after weaning. On d 8, feeders were emptied and a common Phase 2 diet was fed until d 20. Data were analyzed using the GLIMMIX procedure of SAS version 9.1.3 (SAS Institute Inc., Cary, NC) with pen as the experimental unit. Fixed effects used in the model were diet source, vaccination timing, and their interaction. Weight block was included as a random effect. Results are reported as least squares means.

^{abc} Within a row, means without a common superscript letter differ ($P < 0.05$).

Table 3.4 Means for the effect of vaccination timing on nursery pig performance¹ (Exp. 1)

Item	Vaccination timing, d		SEM	Probability, <i>P</i> <
	0	8		
d 0 to 4				
ADG, g	182	193	9.2	0.20
ADFI, g	116	116	7.8	0.94
G:F	1.68	1.67	0.046	0.97
d 4 to 8				
ADG, g	199	227	10.6	0.004
ADFI, g	217	251	12.2	0.01
G:F	0.81	0.91	0.042	0.12
d 0 to 8				
ADG, g	191	210	7.7	0.003
ADFI, g	167	183	7.0	0.01
G:F	1.15	1.15	0.020	0.79
d 8 to 20				
ADG, g	321	307	9.6	0.05
ADFI, g	424	419	14.9	0.44
G:F	0.76	0.74	0.015	0.12
d 0 to 20				
ADG, g	269	268	7.9	0.88
ADFI, g	321	324	11.0	0.60
G:F	0.84	0.83	0.011	0.40
Weight, kg				
d 0	5.7	5.7	0.27	0.46
d 4	6.4	6.5	0.30	0.15
d 8	7.2	7.4	0.32	0.003
d 20	11.0	11.0	0.42	0.78

¹A total of 400 weanling pigs were used in a 20-d growth trial to evaluate the effects of segregated early wean and transition diet source (A, B, C, and D) and vaccination timing (d 0 or 8). There were 5 pigs per pen and 40 pens per vaccination timing treatment. Vaccination timing refers to the day of vaccination. On either d 0 or d 8, pigs were vaccinated with 2 mL Circumvent PCV (Intervet/Schering-Plough Animal Health, Millsboro, DE) and 2 mL RespiSure One (Pfizer Animal Health, New York, NY). Data were analyzed using the GLIMMIX procedure of SAS version 9.1.3 (SAS Institute Inc., Cary, NC) with pen as the experimental unit. Fixed effects used in the model were diet source, vaccination timing, and their interaction. Weight block was included as a random effect. Results are reported as least squares means.

Table 3.5 Means for the effect of circovirus vaccination on nursery pig growth performance, feed intake, and feed efficiency¹ (Exp. 2)

Item	Circovirus vaccine treatment ²			SEM	Probability, <i>P</i> <
	Control	Circumvent PCV	CircoFLEX		
d 0 to 8					
ADG, g	127	117	131	7.1	0.26
ADFI, g	126 ^{ab}	118 ^a	133 ^b	5.1	0.05
G:F	0.99	0.99	0.98	0.027	0.94
d 8 to 14					
ADG, g	333	309	318	13.0	0.10
ADFI, g	436 ^a	396 ^b	432 ^a	17.1	0.03
G:F	0.77	0.78	0.74	0.015	0.13
d 14 to 21					
ADG, g	470	467	462	14.1	0.81
ADFI, g	701	673	698	19.5	0.12
G:F	0.67 ^{ab}	0.69 ^a	0.66 ^b	0.011	0.05
d 21 to 29					
ADG, g	485 ^a	433 ^b	498 ^a	12.9	< 0.001
ADFI, g	771 ^a	712 ^b	781 ^a	18.3	< 0.001
G:F	0.63	0.61	0.64	0.010	0.08
d 29 to 35					
ADG, g	680	672	679	19.9	0.88
ADFI, g	997	980	1,023	26.5	0.10
G:F	0.68	0.69	0.66	0.008	0.13
d 0 to 35					
ADG, g	405 ^a	387 ^b	407 ^a	9.5	0.02
ADFI, g	587 ^a	560 ^b	597 ^a	13.5	0.003
G:F	0.69	0.69	0.68	0.005	0.30
Weight, kg					
d 0	5.9	5.9	5.9	0.26	0.37
d 21	12.2	11.9	12.1	0.43	0.29
d 35	20.1 ^a	19.5 ^b	20.1 ^a	0.54	0.01

¹A total of 360 barrows were used in a 35-d study. At weaning (d 0), barrows were ranked by body weight and assigned to pens. Within weight blocks, pens of pigs were randomly allotted treatments in a 3 × 2 factorial arrangement with main effects of circovirus vaccine and *Mycoplasma hyopneumoniae* vaccine. There were 5 pigs per pen and 24 pens per circovirus vaccine treatment. Data were analyzed using the GLIMMIX procedure of SAS version 9.1.3 (SAS Institute Inc., Cary, NC) with pen as the experimental unit. The model included the fixed effects of circovirus vaccine, *M. hyo* vaccine, and their interaction, and a random effect

of weight block. Results are reported as least squares means.

²Circovirus vaccine treatments were: (1) Control: non-circovirus-vaccinated pigs; (2) Circumvent PCV: 2 mL per dose of Circumvent PCV (Intervet/Schering-Plough Animal Health, Millsboro, DE) injected intramuscularly on d 0 and 21; and (3) CircoFLEX: 1 mL Ingelvac CircoFLEX (Boehringer Ingelheim Vetmedica, Inc, St. Joseph, MO) injected intramuscularly on d 0.

^{ab} Within a row, means without a common superscript differ ($P < 0.05$).

Table 3.6 Means for the effect of *Mycoplasma hyopneumoniae* (*M. hyo*) vaccination on nursery pig growth performance, feed intake, and feed efficiency¹ (Exp. 2)

Item	<i>M. hyo</i> vaccine treatment ²		SEM	Probability, <i>P</i> <
	Control	RespiSure		
d 0 to 8				
ADG, g	128	122	6.1	0.44
ADFI, g	128	124	4.5	0.40
G:F	0.99	0.99	0.022	0.91
d 8 to 14				
ADG, g	312	327	12.3	0.10
ADFI, g	420	423	15.9	0.82
G:F	0.75	0.78	0.012	0.07
d 14 to 21				
ADG, g	476	457	13.2	0.05
ADFI, g	698	684	18.6	0.25
G:F	0.68	0.67	0.010	0.18
d 21 to 29				
ADG, g	485	459	11.6	0.02
ADFI, g	776	734	17.3	0.001
G:F	0.62	0.63	0.009	0.85
d 29 to 35				
ADG, g	685	670	18.5	0.31
ADFI, g	1,018	982	25.2	0.03
G:F	0.67	0.68	0.007	0.28
d 0 to 35				
ADG, g	405	395	9.1	0.10
ADFI, g	589	573	12.8	0.06
G:F	0.69	0.69	0.004	0.62
Weight, kg				
d 0	5.9	5.9	0.26	0.22
d 21	12.1	12.0	0.42	0.50
d 35	20.1	19.7	0.53	0.06

¹A total of 360 barrows were used in a 35-d study. At weaning (d 0), barrows were ranked by body weight and assigned to pens. Within weight blocks, pens of pigs were randomly allotted treatments in a 3 × 2 factorial arrangement with main effects of circovirus vaccine and *M. hyo* vaccine. There were 5 pigs per pen and 36 pens per circovirus vaccine treatment. Data were analyzed using the GLIMMIX procedure of SAS version 9.1.3 (SAS Institute Inc., Cary, NC) with pen as the experimental unit. The model included the fixed effects of circovirus vaccine, *M. hyo* vaccine, and their interaction, and a random effect of weight block. Results are reported as least squares means.

²*M. hyo* vaccine treatments were: (1) Control: non-*M. hyo*-vaccinated pigs; and (2) 2 mL per dose of RespiSure (Pfizer Animal Health, New York, NY) injected intramuscularly on d 0 and 21.

CHAPTER 4 - Effect of vaccination program for porcine circovirus type 2 and *Mycoplasma hyopneumoniae* on commercial pig growth performance, mortality, and carcass characteristics

Summary

Objective(s): To determine, under field conditions, the effect of vaccination program for porcine circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae* (*M. hyo*) on commercial pig growth performance, mortality, and carcass characteristics.

Materials and methods: A total of 1,993 weanling pigs (25.2 ± 1.24 d of age; 7.4 ± 1.70 kg BW) were ranked by birth weight within litter and gender and randomly allotted to different vaccination programs (BI: a 1-dose, two vaccine program, or IN: a 2-dose, two vaccine program), each consisting of vaccines for PCV2 and *M. hyo*. The BI program (Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) included two, 1-dose vaccines: CircoFLEX and MycoFLEX (both 1 mL per dose) administered on d 0. The IN program (Intervet/Schering-Plough Animal Health, Millsboro, DE) included two, 2-dose vaccines: Circumvent PCV (2 mL per dose) and Myco Silencer ONCE (1 mL per dose) administered on d 0 and 23. Pigs of both vaccination programs were comingled within pens. Pigs were weighed on d 0 (weaning), 23, 45, 73, and 155 (off-test) to measure ADG. Data from 1,993 pigs were used for the mortality analysis while data from 1,657 pigs with complete growth records were used for the growth data analysis. Carcass data were analyzed from a subsample of 420 pigs harvested on a single day.

Results: Overall, gilts had decreased ($P < 0.001$) ADG and were lighter ($P < 0.001$) and leaner ($P < 0.001$) than barrows. Vaccination program did influence growth patterns. Pigs vaccinated using the 2-dose, two vaccine IN program had decreased ($P < 0.001$) ADG from d 0 to 73 (nursery), but tended to have increased ($P = 0.06$) ADG from d 73 to 155 (finishing) compared with pigs vaccinated using the 1-dose, two vaccine BI program. Overall, ADG, mortality, and carcass characteristics were similar ($P \geq 0.14$) between the vaccination programs.

Implications: Though there were differences in growth pattern, vaccinating pigs using the BI or IN vaccination program for PCV2 and *M. hyo* resulted in similar postweaning mortality percentages, growth performance, and carcass characteristics.

Key words: growth, *Mycoplasma hyopneumoniae*, PCV2, pig, vaccine

Porcine circovirus type 2 and *M. hyo* are significant pathogens which are endemic in many herds. Porcine circovirus type 2 can cause porcine circoviral disease (PCVD) which manifests as a variety of clinical signs and syndromes in nursery and finishing pigs (Segalés et al., 2005) including pneumonia, diarrhea, death, porcine dermatitis and nephropathy syndrome, and a multi-systemic wasting syndrome. Primary clinical signs of *M. hyo* include pneumonia and cough (Thacker, 2001) which, if severe, can result in production losses.

Commercial vaccines have been available for *M. hyo* for more than 20 years while vaccines for PCV2 were licensed for use beginning less than 5 years ago. Although it is typical standard practice in most United States production systems to vaccinate pigs at or around the time of weaning with vaccines for *M. hyo* and PCV2, there has been little research evaluating these combinations under field conditions. Also, while vaccination against PCV2 and *M. hyo* has been effective for attenuation of clinical disease or lesions during finishing, in some studies vaccination for PCV2 or *M. hyo* negatively affected nursery pig performance immediately after vaccination (Kane et al., 2009; Shelton et al., 2009). In a different study, these effects were product-dependent with certain vaccines having no negative effects on performance after vaccination (Potter et al., 2009). These studies were limited to the nursery period (Kane et al., 2009; Potter et al., 2009) or were performed under disease-conditions where vaccination improved subsequent performance compared with non-vaccinated controls (Shelton et al., 2009). Therefore, it was not determined whether the negative effects on growth rate experienced after vaccination with specific products were maintained through finishing or whether finishing performance varied with vaccine.

Therefore, the objective of this field study was to compare the effects of different vaccination programs for PCV2 and *M. hyo* on commercial pig growth performance, mortality, and carcass characteristics.

Materials and Methods

Procedures used in this study were approved by the Kansas State University Institutional Animal Care and Use Committee. This study was performed in a commercial farm in northeast Kansas. Nursery and finisher pens were equipped with a dry, self-feeder and a cup waterer which allowed all pigs to have ad libitum access to feed and water. The herd was positive for *M. hyo* and historically had experienced clinical PCVD. Diagnosis of PCVD had been based on evidence of gross abnormalities at necropsy with lesion presence confirmed by histologic evaluation. Immunohistochemistry was performed and demonstrated PCV2 antigen in tissues. After implementation of a circovirus vaccination program, clinical signs of PCVD were mitigated.

Pigs born over a 22 d period in a single sow farm were used in this 155-d field trial. At birth, all pigs were individually weighed and identified by a unique numbered ear-tag. Records for pre-weaning mortalities were not maintained.

At weaning (d 0), a total of 1,993 pigs (25.2 ± 1.24 d of age; 7.4 ± 1.70 kg BW) representing 213 litters of 2 genetic backgrounds (PIC 327 \times Triumph TR24 or PIC 327 \times PIC 1050) were ranked by birth weight within litter and gender (barrow or gilt) and randomly assigned to different vaccination programs (BI or IN). Each program included two vaccines, one for *M. hyo* and the other for circovirus, which were produced by a single manufacturer.

Vaccination programs were either: (1) BI: a 1-dose, two vaccine program (vaccines manufactured by Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) with Ingelvac CircoFLEX and Ingelvac MycoFLEX, or (2) IN: a 2-dose, two vaccine program (vaccines manufactured by Intervet/Schering-Plough Animal Health, Millsboro, DE) with Circumvent PCV and Myco Silencer ONCE. CircoFLEX and Circumvent were the vaccinations for circovirus while MycoFLEX and Myco Silencer ONCE were the vaccinations for *M. hyo*. For the BI vaccination program, unopened bottles of CircoFLEX and MycoFLEX were mixed (50:50) on each day of administration (d 0) and a 2 mL volume of the mixed solution (1 mL CircoFLEX and 1 mL MycoFLEX) was administered as a single intramuscular injection. Vaccines for the IN vaccination program were administered as separate intramuscular injections (Circumvent PCV: 2 mL per dose; and Myco Silencer ONCE: 1 mL per dose) on d 0 and 23.

Pigs were weaned twice a week and were individually weighed and placed consecutively into pens in one of four 500-head nursery rooms over 6 weaning days. There were 25 pigs per

pen and a room was filled completely before starting to fill the next nursery room. Pigs of both vaccination programs were comingled within pens. Pigs were individually weighed by weaning group on d 0 (weaning), and then were weighed by nursery and finishing room resulting in average weigh days of d 23 (range: d 20 to 28), d 45 (range: d 41 to 49), d 73 (range: d 68 to 79), and d 155 (range: d 132 to 163). Pigs were vaccinated on d 0 (BI and IN) and d 23 (IN only) in accordance with their assigned vaccination program. Immediately after vaccination, pigs were monitored for a “fainting” reaction. This reaction consisted of convulsion-like activity including collapsing, immobility, exhibition of involuntary muscle contractions, and irregular respiration. Pigs appeared to recover within a short amount of time and there were no pigs that died during the reaction period in this study.

Pigs were moved from the nursery rooms to a single finishing barn with multiple rooms and penned with 30 pigs per pen. Pigs representing both vaccination programs were again comingled within pens. Pigs within a finishing room were weighed off test on a single day. Pigs that weighed 97.5 kg or greater met the minimum weight criteria for the packing plant and were marketed in accordance with standard farm procedures. Carcass data were collected on a subsample of 420 pigs from one finishing room. Pigs were randomly selected to have a similar weaning age (24.9 ± 1.32 d of age) and weight distribution (7.5 ± 1.65 kg BW) as the whole population. Pigs for carcass data collection were weighed off test and individually tattooed on a single day and then marketed on a single day (d 167). A total of 101 BI program-vaccinated barrows, 113 BI program-vaccinated gilts, 102 IN program-vaccinated barrows, and 104 IN program-vaccinated gilts were represented in this subsample. Carcass data collected on the subsample of pigs included hot carcass weight, backfat depth, loin depth, and lean percentage.

Mortalities were recorded throughout the trial. Records from these pigs as well as from pigs which were unidentifiable because of lost ear tags, or pigs that were missing weight records were not used in the growth data analysis. There were 61 pigs that died, 259 pigs missing ear tags, and 16 pigs with incomplete growth records. Statistical analyses for mortality and pigs missing data was performed on individual records from 1,993 pigs while analysis for growth performance were performed on individual records from 1,657 pigs which had complete growth records at the end of the trial. Statistical analyses for carcass traits were performed on individual records from 420 pigs.

Analysis:

Growth, mortality, and carcass data were analyzed by analysis of variance using the MIXED procedure in SAS version 9.1.3 (SAS Institute Inc., Cary, NC) to obtain least squares means and standard errors for all response criteria. The statistical model included the fixed effects of vaccination program, gender, and their interaction. Litter of origin was included as a random effect. Individual pig was the experimental unit for all analyses. Individual pig average daily gain (ADG) was calculated by dividing the period weight gain by the number days in the period. For carcass data analysis, loin depth, backfat depth, and lean percentage were adjusted to a common average hot carcass weight.

Significance tests were performed for comparisons of the means of fixed effects and their interactions. Values of $P < 0.05$ were considered significant for these analyses.

Results

There were no 2-way interactions ($P \geq 0.23$) between gender and vaccination program observed for any age, growth, carcass, or mortality responses. A similar percentage of barrow and gilts died (barrow: 3.6 ± 0.66 % vs. gilt: 3.5 ± 0.64 %; $P = 0.92$) during the trial. Similarly, vaccination program did not affect mortality (BI: 3.9 ± 0.65 % vs. IN: 3.3 ± 0.65 %; $P = 0.49$). A similar percentage of records were removed from analysis because of lost ear tags and incomplete growth records between the genders (barrow: 15.1 ± 1.29 % vs. gilt: 13.6 ± 1.28 %; $P = 0.33$) and the vaccination programs (BI: 15.5 ± 1.27 % vs. IN: 13.2 ± 1.28 %; $P = 0.11$).

A similar ($P = 0.23$) percentage of barrows and gilts exhibited a “fainting” reaction following vaccination. However, vaccination program did affect ($P < 0.001$; Table 4.1) the percentage of pigs that showed signs of the “fainting” reaction (a reaction consisting of convulsion-like activity) after vaccination. The reaction was exhibited by a larger percentage of pigs of the IN-vaccination program than that of the BI-vaccination program.

Mean ages were similar ($P = 0.59$, data not shown) between barrows and gilts at each of the 5 time points when pigs were weighed. From d 0 to 23, gilts grew faster ($P = 0.01$) than barrows. From d 23 to 45 and from d 0 to 45 there was no difference ($P \geq 0.24$) in growth rates between the genders. Barrows grew faster ($P < 0.001$) than gilts from d 45 to 73 and from d 0 to 73 (nursery period). The late nursery increase in ADG for barrows led to heavier ($P < 0.001$) d 73 weights for barrows compared with gilts.

From d 73 to 155 (finishing period), barrows continued to have increased ($P < 0.001$) ADG compared with gilts. Thus, overall d 0 to 155 growth rate was increased ($P < 0.001$) for barrows compared with gilts. On d 155, barrows were 10.6 kg heavier ($P < 0.001$) than gilts. A greater ($P < 0.001$) percentage of gilts were unmarketable due to light weight at the end of the trial compared with barrows.

Hot carcass weight was increased ($P < 0.001$) for barrows compared with gilts. After adjustment to a common average HCW, barrows had increased ($P < 0.001$) backfat depth, decreased ($P < 0.001$) loin depth, and lower ($P < 0.001$) percentage lean compared with gilts.

Mean ages were similar ($P = 0.13$, data not shown) between pigs of the IN vaccination program and those of the BI vaccination program at the 5 time points for weight data collection. Following the first vaccination (d 0 to 23), there was no difference ($P = 0.48$) observed for pig growth rate between the 2 vaccination programs. However, after pigs of the IN vaccination program had received the second dose of both vaccines, pigs of the BI vaccination program had increased ($P < 0.001$) ADG compared with IN-program vaccinated pigs from d 23 to 45 and d 0 to 45. The difference in growth rate resulted in lighter ($P = 0.001$) d 45 weights for pigs vaccinated using the IN vaccination program compared with pigs vaccinated using the BI vaccination program. Pigs vaccinated using the BI vaccination program continued to have increased ($P = 0.04$) ADG from d 45 to 73 compared with pigs vaccinated using the IN vaccination program. For the overall nursery period (d 0 to 73) pigs vaccinated using the BI vaccination program had increased ($P < 0.001$) growth rate compared with that of the pigs vaccinated using the IN vaccination program. On d 73, the end of the nursery period, pigs vaccinated using the BI vaccination program were 1.1 kg heavier ($P = 0.001$) than pigs vaccinated using the IN vaccination program.

Throughout the finishing period, from d 73 to 155, pigs vaccinated using the BI vaccination program tended ($P = 0.06$) to have decreased ADG compared with that of pigs vaccinated using the IN vaccination program. Overall (d 0 to 155) there was no difference ($P = 0.83$) in pig growth rate between the vaccination programs. The similar overall growth rates resulted in no difference ($P = 0.66$) in d 155 (off-test) weights of pigs regardless of whether pigs were vaccinated using the BI vaccination program or IN vaccination program. There was no difference ($P = 0.67$) in cull and light-weight (less than 97.5 kg) pig percentages between vaccination programs.

There was no difference ($P \geq 0.14$) in HCW, adjusted backfat depth, adjusted loin depth, or adjusted lean percentage between pigs of either vaccination program.

Discussion

Vaccination against circovirus or *M. hyo* either reduces lesion severity or prevents active infection resulting in fewer clinical signs of disease (Baccaro et al., 2006; Horlen et al., 2008; Jacela et al., 2011; Jensen et al., 2002; Rapp-Gabrielson et al., 2008). Despite *M. hyo* vaccines having been commercially available longer than circovirus vaccines, few independent, refereed studies have been published which compare 1-dose and 2-dose *M. hyo* products.

Considerations when selecting a 1-dose or 2-dose vaccination protocol may be based on level of compliance to vaccinate. With questionable or low compliance, implementing a 2-dose vaccination program could help to ensure more pigs are exposed to some amount of vaccine. There are limited available data comparing the efficacy of 1-dose and 2-dose *M. hyo* vaccines. However, some studies have indicated that efficacy was, at a minimum, comparable between 1-dose and 2-dose *M. hyo* vaccines (Morris and Sanford, 2001; Roof et al., 2001).

Roof et al. (2001) evaluated lung lesion severity in non-vaccinated control pigs compared with pigs vaccinated with 1 of 4 commercial *M. hyo* vaccines: (1) Ingelvac M. hyo: 1-dose (Boehringer Ingelheim Vetmedica, Inc., Canada), (2) Ingelvac M. hyo: 2-dose (Boehringer Ingelheim Vetmedica, Inc., Canada), (3) RespiSure One: 1-dose (Pfizer Animal Health, New York, NY), and (4) RespiSure: 2-dose (Pfizer Animal Health, New York, NY). It was reported that after *M. hyo* challenge, with an exception for the 2-dose RespiSure product, all vaccines were effective at reducing lung lesion severity in vaccinated pigs compared with non-vaccinated controls (Roof et al., 2001). In a different study performed under field conditions, there was no difference in lung lesion severity between pigs vaccinated with a 1-dose *M. hyo* vaccine (Ingelvac M. hyo) and a 2-dose vaccine (RespiSure) (Morris and Sanford, 2001).

Although lesion severity was often reduced in pigs vaccinated against *M. hyo* compared with non-vaccinates, growth responses to *M. hyo* vaccines were not consistent between trials (Maes et al., 1999; Morrow et al., 1994). Also, it has not been well defined whether the growth responses previously reported for specific vaccines would be representative of vaccine responses achieved under current production practices.

Circovirus vaccines have been more recently evaluated than *M. hyo* vaccines as the first circovirus vaccine became available in 2006 for use in the United States. Despite the initial research emphasis on circovirus vaccines, at the time of our trial, there were few side-by-side comparisons of 1-dose and 2-dose circovirus vaccine products and their effects on wean-to-finish pig performance under field conditions. In one field study, though there were numeric differences, pigs vaccinated with a 2-dose circovirus vaccine (Circumvent PCV) had similar growth rates and off-test weights compared with pigs vaccinated with a 1-dose circovirus vaccine (Suvaxyn PCV2 One Dose; Fort Dodge Animal Health, Fort Dodge, IA). Both vaccinated groups had improved growth performance compared with controls (Jacela, 2009). In several other separate field studies, 1-dose or 2-dose circovirus vaccines were found to be effective at reducing lesions, viremia, mortality, or increasing finishing growth rates in vaccinated pigs when compared with non-circovirus-vaccinated control pigs (Fachinger et al., 2008; Horlen et al., 2008; Jacela et al., 2011; Kixmüller et al., 2008).

Although finishing performance can be improved by the use of circovirus vaccines, some circovirus and *M. hyo* vaccine products have been shown to negatively affect nursery pig performance. One study indicated that within the 8 d after initial vaccination, pigs vaccinated with Circumvent PCV and RespiSure One had decreased ADG, ADFI, and lighter d 8 weights compared with non-vaccinated control pigs (Kane et al., 2009). In a follow-up study, pigs vaccinated with Circumvent PCV had reduced ADG and ADFI compared to non-vaccinated control pigs or pigs vaccinated with CircoFLEX. There were no differences in performance between the control and CircoFLEX-vaccinated pigs in the 35 d study. Pigs vaccinated with the 2-dose RespiSure vaccine had lower ADG compared with non-vaccinated control pigs from d 14 to 21 and 21 to 25. On d 35, pigs vaccinated with RespiSure tended to weigh less than control pigs (Potter et al., 2009). Thus, these studies indicated the effects after vaccination were product-dependent and these effects should be considered when developing vaccination protocols. However, other factors must also be considered when developing vaccination protocols including timing of vaccination in relation to pathogen exposure, labor and product costs, and product efficacy for the intended purpose.

Our study was designed to compare 2 commonly used circovirus and *M. hyo* vaccination programs. Each program had vaccines supplied by a single manufacturer. The 1-dose program evaluated was the BI program with vaccines: MycoFLEX and CircoFLEX. Each of these

vaccines contained an adjuvant, ImpranFLEX (Roof, 2010), which was a proprietary aqueous-based polymer. This adjuvant did not contain mineral oil. There has been suggestion that an adjuvant without mineral oil may be better tolerated by an animal. At the time of our study, MycoFLEX and CircoFLEX had recently received a label for combined administration as a single injection. This increased the convenience of this program as a single 2 mL injection (1 mL MycoFLEX and 1 mL CircoFLEX) was administered to each pig.

The 2-dose, two vaccine program evaluated was the IN program with vaccines: Myco Silencer ONCE and Circumvent PCV. Myco Silencer ONCE had a dual label for administration as a single 2 mL dose or as a split 2-dose (1 mL per dose) administration. We administered the vaccine using the 2-dose regimen as that was the protocol being used by the production system at the time of our study. Therefore, a total of 2 injections, 1 mL Myco Silencer ONCE and 2 mL Circumvent PCV, were administered twice for this second vaccination program. Each of these vaccines contained the adjuvant, Microsol Diluvac Forte (Thacker, 2006), a proprietary dual oily emulsion adjuvant. Results from our study indicate that pigs vaccinated using the 2-dose, two vaccine IN program had decreased growth performance from d 0 to 73 compared to the pigs vaccinated using the BI program. The majority of this decrease in performance occurred after administration of the second dose of IN vaccines. Previous reports supported these findings (Potter et al., 2009; Shelton et al., 2009). It could not be determined whether the BI vaccination program had any negative effect on the growth rate of the weaned pig as non-vaccinated control pigs were not included in the study. At the time our study was designed, pigs left not vaccinated for PCV2 in this commercial system were at risk for PCV2 infection and production loss.

In contrast to the d 0 to 73 results, from d 73 to 155 growth rates effects were reversed. Pigs vaccinated with the 2-dose IN vaccines tended to have improved performance compared with pigs vaccinated with BI vaccines. These growth rate differences resulted in pigs being of similar average off-test weight between vaccination programs. In addition, vaccination program did not affect percentage of cull and light-weight pigs.

Historically *M. hyo* and PCV2 affected pigs primarily during the finishing period, thus, it was our speculation that these late growth rate differences occurred as a result of differences in immunity. It was not possible to separate the effects of the circovirus and *M. hyo* vaccination nor do we know the level of pathogen exposure which occurred during our study. However, in a field study using a *M. hyo*-free herd, Vilaca et al. (2010) reported growth performance patterns

which were similar to those observed in our study which was performed in a *M. hyo*-positive herd. In their study, pigs were assigned to either a 1-dose circovirus vaccination (1 mL per dose) treatment, a 2-dose vaccination (2 mL per dose) treatment, or a saline-injected control treatment. Their results indicated a decrease in nursery performance associated with the 2-dose circovirus vaccination compared with the 1-dose vaccination treatment and the controls. During the finishing period in their study, growth rates were increased for the pigs vaccinated with the 2-dose product compared with pigs vaccinated with the 1-dose vaccine or the control pigs. Growth rates were also greater for pigs in the 1-dose vaccinated treatment compared with the control pigs. Viremia was confirmed in the study by PCV2 polymerase chain reaction (Vilaca et al., 2010).

For our study, the overall growth performance, mortality, and carcass characteristics were not different between the 2 circovirus and *M. hyo* vaccination programs. These data would suggest that either program would result in the same outcome for producers who own pigs from weaning to market. However, these results may especially be of interest to those who buy or sell pigs prior to the finishing period. Nevertheless, further investigation of different vaccination programs may be warranted to fully explain the resulting differences in growth performance patterns. Although growth rates varied by period between the vaccination programs, in this commercial herd, the circovirus and *M. hyo* vaccination program used did not affect overall pig performance, mortality, or carcass characteristics.

Implications

- The 1-dose, two vaccine (BI; CircoFLEX and MycoFLEX) or 2-dose, two vaccine (IN; Circumvent PCV and Myco Silencer ONCE) vaccination program for circovirus and *M. hyo* resulted in similar overall growth performance despite differences in pattern of growth rate.
- Growth performance from d 0 to 73 (nursery period) was improved by the BI vaccination program while d 73 to 155 (finishing period) growth performance tended to be improved by the IN vaccination program.
- Mortality and carcass characteristics were not different between the 1-dose, two vaccine program or the 2-dose, two vaccine program for vaccination against porcine circovirus type 2 and *M. hyo*.

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Table 4.1 Effect of gender or vaccination program on reactions, performance, light-weight pig percentages, and carcass traits¹

Item	Gender		Vaccination program ²		Probability, <i>P</i> <	
	Barrow	Gilt	BI	IN	Gender	Program
"Fainting" reaction, % ³	1.1 ± 0.32	0.6 ± 0.31	0.0 ± 0.32	1.7 ± 0.32	0.23	<0.001
ADG, g						
D 0 to 23	334 ± 4.4	343 ± 4.3	337 ± 4.3	340 ± 4.3	0.01	0.48
D 23 to 45	643 ± 5.7	637 ± 5.7	656 ± 5.7	623 ± 5.7	0.24	<0.001
D 0 to 45	485 ± 4.6	487 ± 4.5	493 ± 4.5	479 ± 4.5	0.68	<0.001
D 45 to 73	746 ± 6.1	676 ± 6.0	718 ± 6.0	704 ± 6.0	<0.001	0.04
D 0 to 73 ⁴	587 ± 4.6	561 ± 4.5	581 ± 4.5	567 ± 4.5	<0.001	<0.001
D 73 to 155 ⁵	915 ± 5.3	811 ± 5.3	857 ± 5.3	868 ± 5.3	<0.001	0.06
D 0 to 155 ⁶	761 ± 4.2	694 ± 4.2	728 ± 4.2	727 ± 4.2	<0.001	0.83
Weight, kg						
D 0 (weaning)	7.6 ± 0.08	7.5 ± 0.08	7.5 ± 0.08	7.5 ± 0.08	0.11	0.57
D 23	15.2 ± 0.16	15.2 ± 0.16	15.2 ± 0.16	15.2 ± 0.16	0.56	0.92
D 45	29.1 ± 0.27	29.0 ± 0.27	29.4 ± 0.27	28.7 ± 0.27	0.82	0.001
D 73	50.3 ± 0.39	48.3 ± 0.38	49.8 ± 0.38	48.7 ± 0.38	<0.001	0.001
D 155 (off-test)	125.7 ± 0.71	115.1 ± 0.70	120.6 ± 0.70	120.3 ± 0.70	<0.001	0.66
Culls and < 97.5 kg BW, % ⁷	5.9 ± 1.10	11.4 ± 1.08	8.3 ± 1.08	8.9 ± 1.08	<0.001	0.67
Carcass characteristics ⁸						
HCW, kg	97.4 ± 0.84	91.2 ± 0.83	93.9 ± 0.82	94.7 ± 0.83	<0.001	0.41
Backfat depth, mm ⁹	19.8 ± 0.47	17.1 ± 0.47	18.2 ± 0.46	18.7 ± 0.47	<0.001	0.14
Loin depth, mm ⁹	55.5 ± 0.49	58.4 ± 0.48	56.7 ± 0.47	57.2 ± 0.48	<0.001	0.41
Lean percentage, % ⁹	51.8 ± 0.18	53.3 ± 0.18	52.6 ± 0.18	52.5 ± 0.18	<0.001	0.59

¹ A total of 1,993 weanling pigs (initially 25.2 ± 1.24 d of age; 7.4 ± 1.70 kg BW) were used in a wean-to-finish growth trial to evaluate the effects of gender (barrow or gilt) and circovirus and *Mycoplasma hyopneumoniae* vaccination program (BI: a 1-dose,

two vaccine program, or IN: a 2-dose, two vaccine program) on pig performance. Weaning occurred twice a week and pigs consecutively placed in 4 nursery rooms were started on test over 6 weaning days. Pigs were then weighed by nursery or finishing room resulting in average weigh days of d 23, 45, 73, and 155 (off-test). Growth data analysis was performed on records from 1,657 pigs (initially 25.1 ± 1.22 d of age) which had complete growth records at off-test. Data for the gender effect were from 805 barrows and 852 gilts while data for the effect of vaccination program were from 820 BI-program vaccinated pigs and 837 IN-program vaccinated pigs. Results were reported as least squares means \pm SEM.

² Pigs assigned to the 1-dose, two vaccine BI vaccination program received 1 mL Ingelvac CircoFLEX (Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) and 1 mL Ingelvac MycoFLEX (Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) as a single intramuscular injection on d 0. Pigs assigned to the 2-dose, two vaccine IN vaccination program received 2 mL Circumvent PCV (Intervet/Schering-Plough Animal Health, Millsboro, DE), and 1 mL Myco Silencer ONCE (Intervet/Schering-Plough Animal Health, Millsboro, DE) as separate intramuscular injections on d 0 and 23.

³ "Fainting" reaction exhibited after vaccination characterized by immediate convulsion-like activity.

⁴ Nursery ADG.

⁵ Finisher ADG.

⁶ Wean-to-finish ADG.

⁷ Percentage of pigs considered to be culls or weigh less than 97.5 kg (minimum weight for the packing plant) on d 155.

⁸ Carcass data were collected on a subset of 420 pigs (203 barrows and 217 gilts; 214 BI-program vaccinated and 206 IN-program vaccinated pigs) harvested on a single day from a single finishing room. Pigs were randomly selected to have a similar weaning age (24.9 ± 1.32 d of age) and weight distribution (7.5 ± 1.65 kg BW) as the whole population.

⁹ Carcass traits were adjusted to a common average HCW.

CHAPTER 5 - Utilizing vaccination for porcine circovirus type 2 (PCV2) as a tool to aid elimination of PCV2 from swine populations

Abstract

Objective: To determine whether circovirus vaccination influenced porcine circovirus type 2 (PCV2) circulation within a herd and could be used as a tool to eliminate PCV2 from PCV2-positive swine herds.

Design: Phases 1 and 5 used a cross-sectional and phases 2, 3, and 4 used a longitudinal design.

Animals: A total of 928 pigs from the Michigan State University (MSU) Swine Teaching and Research Center, Kansas State University (KSU) Swine Teaching and Research Center, and a Kansas commercial farm were used during a 3-year monitoring study.

Procedures: Infection with PCV2 was confirmed by polymerase chain reaction (PCR) and indirect fluorescent antibody (IFA) testing in both university herds before introduction of circovirus vaccine. After vaccination implementation, vaccinated barrows were selected from consecutive weaning groups and serially bled for detection of viremia. Follow-up testing with circovirus-vaccinated and non-vaccinated pigs was performed at the KSU farm. Pigs were individually weighed and a subsample bled 4 times for antibody detection. In a circovirus-vaccinated commercial herd, serial-bleeding and PCV2 PCR testing on serial serum samples from 85 non-circovirus-vaccinated pigs was completed. Testing by PCV2 PCR was performed on environmental swab samples collected from facilities at the KSU and commercial farms.

Results: Sera from 0 of 9 MSU vaccinated-cohorts and 3 of 10 KSU vaccinated-cohorts had detectable PCV2 DNA. Of the three KSU cohorts that had detectable PCV2 DNA, only one group had more than one sample with detectable PCV2 DNA. From follow-up testing, a PCV2 antibody rise after vaccination was detected for vaccinated pigs with no detectable rise in antibody for the non-vaccinated pigs. Overall growth rate of non-vaccinated pigs tended ($P = 0.07$) to be increased compared with vaccinated pigs. Antibody and growth results indicate a

lack of PCV2 exposure at the KSU farm. In contrast, non-vaccinated pigs did become PCV2 viremic at the commercial farm. Viral DNA was detected in the environment of the commercial farm but not from the KSU facilities.

Conclusions and Clinical Relevance: Circovirus vaccination affected PCV2 circulation in the university herds that appeared to have low initial viral exposure. However, viral circulation was unaffected in a commercial herd where environmental PCV2 viral sources were detected. Circovirus vaccine provided a tool to affect viral circulation on farms but would need to be used in conjunction with other management practices to eliminate PCV2 from most swine populations.

Introduction

Porcine circovirus type 2 (PCV2) is a necessary agent for causing porcine circovirus disease (PCVD), a multi-syndrome, production-damaging disease (Segalés et al., 2005). Identified in diagnostic laboratory samples back to the early 1990's, PCV2 has affected most United States swine herds. Despite a long history of PCV2 circulation within the swine population, vaccines against PCV2 have only been commercially-available since 2006 (Opriessnig et al., 2009). Initial studies evaluating the effects of circovirus vaccination on production parameters in PCV2-affected herds indicated that vaccination was effective at reducing finishing phase mortality and increasing pig growth rate (Fachinger et al., 2008; Horlen et al., 2008; Kixmöller et al., 2008). In single-cohort studies, vaccination with commercial or experimental vaccines against PCV2 reduced viremia (Fachinger et al., 2008; Kixmöller et al., 2008) and decreased viral shedding in nasal secretions and feces (Fort et al., 2008; Fort et al., 2009). However, there are limited data evaluating the effects of vaccination on PCV2 viral circulation within a herd over time. Our goal was to monitor PCV2 viral circulation in swine herds after implementing a circovirus vaccination program for growing pigs. The short-term objective of this project was to determine whether circovirus vaccination could be used to affect viral circulation within 2 farrow-to-finish herds. The long-term objective for the project was to understand whether use of circovirus vaccines over time in PCV2-positive swine herds could provide a tool to eliminate PCV2 from these herds.

Materials and Methods

Procedures used in these studies were approved by the Kansas State University and Michigan State University Institutional Animal Care and Use Committees.

Herd History:

The Michigan State University (MSU) and Kansas State University (KSU) Swine Teaching and Research Centers were single-location farrow-to-finish operations. Pigs were moved through the KSU farm in an all-in, all-out manner in nursery, grower, or finisher rooms. In the MSU farm, about half of the pigs placed in a nursery, grower, or finisher room were moved in and out at a time. Pigs were born (farrowed) at each farm approximately every 4 (MSU) or 5 (KSU) wk which resulted in growing pig populations of about 300 pigs in each age group. Both herds were negative for porcine reproductive and respiratory syndrome virus and the MSU herd was negative for *Mycoplasma hyopneumoniae* (*M. hyo*). Pigs at the KSU farm were vaccinated at weaning for *M. hyo* (RespiSure-ONE; Pfizer Animal Health, New York, NY) which, along with other management procedures, contributed to low levels of clinical disease. Prior to the start of our study, both farms had been closed to live animal introductions; however, semen was introduced from outside sources. In October 2007, the KSU farm began to bring replacement gilts from an outside source into the herd approximately every 9 wk.

Clinical History:

The KSU farm did not have any clinical signs of PCVD noted before the baseline testing and subsequent implementation of a circovirus vaccination program. Although prior to baseline testing, histopathologic evaluation on tissues of one pig documented lymphoid depletion lesions consistent with PCVD. The MSU farm had evidence of moderate clinical PCVD (10 to 15% nursery mortality) prior to baseline testing.

Phase 1: Baseline testing procedures

In early 2007, a cross-sectional survey was conducted of each university herd to verify the presence of PCV2 and to characterize patterns of PCV2 infection and seroconversion. At the MSU farm, blood was collected from 101 pigs across a total of 5 growing pig populations (6 to 10, 11 to 15, 16 to 20, 21 to 25, and 26 to 30 wk of age). Within the KSU farm, 141 pigs were sampled across 5 growing pig populations (4, 9, 14, 19, and 24 wk of age). Serum was pooled (MSU: 21 pools, and KSU: 27 pools) within age group and analyzed using the Kansas State

Veterinary Diagnostic Laboratory (KSVDL) PCV2 PCR assay for detection of PCV2 nucleic acid. Viral template quantities for each serum pool were \log_{10} transformed and transformed results were averaged for pools within each age range to characterize the changes in viral load. For the detection of PCV2 antibodies, individual serum samples were tested using the 96-well format KSVDL PCV2 IFA assay with serial 1:2 dilutions beginning with a 1:20 serum to phosphate-buffered saline dilution and ending with a 1:2,560 ratio. The titration endpoint was calculated as the reciprocal of the last serum dilution that gave a positive result. Diagnostic testing methods were accepted and validated in accordance with the American Association of Veterinary Laboratory Diagnosticians' standard requirements necessary for diagnostic laboratory accreditation.

All IFA titers were \log_2 transformed to approximate a normal distribution prior to descriptive analysis. For samples which did not have antibody detected at the most concentrated dilution (1:20), the \log_2 of 10 was used in the analysis. For samples which were considered by the trained IFA technician to be strongly positive at the least concentrated dilution (1:2,560), the \log_2 of 5,120 was used. This allowed these results to be weighted differently than samples with antibody detected with a normal level of fluorescence at the 1:20 and 1:2,560 dilutions.

Infection and antibody profiles obtained from the baseline testing were considered when deciding on sampling times for the Phase 2 study on each farm.

Phase 2: Trial procedures

In the spring of 2007, both MSU and KSU initiated circovirus vaccination programs. A 2-dose circovirus vaccine (Circumvent PCV; Intervet/Schering-Plough, Millsboro, DE) was administered as an intramuscular injection (2 mL per dose) to all growing pigs in each weaning group with 3 to 5 wk between vaccine doses. Pigs were weaned and vaccinated with the first dose of circovirus vaccine at approximately 3 wk of age at the KSU farm while at the MSU farm there was some variation within weaning age or timing of first vaccination (range: 2 to 6 wk).

From 2007 to 2008, barrows from consecutive weaning cohorts at the MSU (9 groups) and KSU (10 groups) farms were monitored for PCV2 viremia. A minimum of 12 barrows per group from different litters were randomly selected, ear-tagged, and serially-bled at 4 time-points: weaning or just before vaccination, entry-to-finishing, mid-finishing, and end-of-finishing. After completion of data collection in 2008, individual serum samples for pigs with complete serum sets (4 serum samples per pig) were tested by the KSVDL PCV2 PCR assay for

detection of PCV2 nucleic acid. An average of 40 cycles was run with a cycle time threshold of 0.05 for classification of PCV2 nucleic acid-containing (positive) samples.

Phase 3: Follow-up monitoring procedures

Beginning in the spring of 2009, a total of 372 pigs (186 non-vaccinated control pigs and 186 circovirus-vaccinated pigs) across 3 weaning groups were used in a Phase 3 growth and PCV2 antibody follow-up study at the KSU farm. At the start of the Phase 3 study, the KSU farm had been vaccinating pigs against PCV2 for the previous 2 years. During that time there had been no evidence of clinical disease. A first objective of this follow-up study was to document the effects of circovirus vaccination on PCV2 antibody titers and to determine whether there was evidence of PCV2 exposure. A second objective of this Phase 3 study was to evaluate the effects of circovirus vaccination on growth rate of pigs in the KSU herd.

Three groups of pigs were used in the Phase 3 study. For groups 1 and 2, there were 7 pigs per nursery pen. A total of 18 barrow pairs (36 pigs; 1 pair in each of 18 pens) for group 1 and 30 barrow pairs (60 pigs; 1 pair in each of 30 pens) for group 2 were utilized. Within a pen, a pair of barrows was selected with one barrow per pair randomly allotted to a vaccinated treatment and the pen-mate barrow assigned to the non-vaccinated control treatment. Barrows assigned to the vaccinated treatment were injected intramuscularly with a 2-dose circovirus vaccine (Circumvent PCV) at approximately 3 and 6 wk of age. All other pigs in the weaning group not enrolled in the follow-up study were vaccinated with the same 2-dose circovirus vaccine.

Throughout the entire study, pairs of barrows remained penned together thereby ensuring the same diets were fed to both vaccinated and non-vaccinated barrows. Each pair also experienced the same environmental and pig exposures. Barrows were individually weighed and bled at 4 time-points: d 0 (pre-vaccination), entry-to-finisher, mid-finisher, and end-of-finisher. From these data, average daily gain (ADG) was calculated for 3 periods: nursery and grower, finisher, and overall nursery to finisher. Removals and mortalities were recorded and weighed and their gain and time on test were included in performance calculations.

For group 3, 138 barrow or gilt pairs (276 pigs) were randomly allotted to treatments (vaccinated or non-vaccinated control) at the time of weaning with procedures similar to those used for groups 1 and 2. However, for group 3, there were 6 or 8 pig per nursery pen (3 of 4 pairs within a pen) and all pigs were placed on test. Pigs assigned to the vaccinated treatment

were injected intramuscularly with a 2-dose circovirus vaccine (Circumvent PCV) at approximately 3 and 9 wk of age. Weighing and penning procedures for each pair were similar to those used for groups 1 and 2. A subset of 20 barrow pairs (40 pigs) from 20 different pens distributed throughout the nursery were bled at the time of weighing. Pairs of barrows were selected and, within each pair, one barrow was randomly assigned to a vaccinated treatment and the pen-mate barrow assigned to the non-vaccinated control treatment. For group 3, removals and mortalities were recorded and weighed and their gain and time on test were included in performance calculations.

Once all serum had been collected, individual serum samples for groups 1, 2, and 3 were tested for PCV2 antibodies using the KSVDL IFA assay. Test procedures used were similar to those used in Phase 1; however, an initial serum to phosphate-buffered saline dilution of 1:40 was used with subsequent serial 1:3 dilutions for groups 1, 2, and 3 samples. To account for variability due to IFA plate, all samples for each pair of pigs were tested on the same IFA plates with the exception of 1 pair which had samples from each bleed tested across 4 IFA plates because of sample space availability. Samples were randomly assigned to plate wells and negative and positive controls were run on each IFA plate. Testing was performed over 7 days (2 days for group 1, 3 days for group 2, and 2 days for group 3) and pairs of pigs were balanced across IFA days within each study.

Group 1, 2, and 3 IFA titers were \log_3 transformed to approximate a normal distribution prior to statistical analysis. For samples which did not have antibody detected at the most concentrated dilution (1:40) the \log_3 of 13.3 was used in the analysis while the \log_3 of 262,440 was used for analysis for samples which were considered by a single trained IFA technician to be strongly positive at the least concentrated dilution (1:87,480). This allowed these samples to be weighted differently than positive samples with normal level fluorescence at 1:40 and 1:87,480.

Group 1, 2, and 3 IFA data were analyzed by repeated measures analysis using the GLIMMIX procedure in SAS version 9.1.3 (SAS Institute, Inc., Cary, NC). Fixed effects in the model included treatment, time, and their interaction. Group and IFA day were used as random effects. Differences between treatments were determined using least squares means ($P < 0.05$). \log_3 transformed least squares means were transformed back to the original scale for presentation as geometric mean titers (GMT).

Growth data were analyzed using the GLIMMIX procedure in SAS version 9.1.3. The interaction with gender and treatment was determined to be non-significant for group 3, and growth data was pooled across the genders for subsequent analysis of the treatment effect. Thus, growth data for all 3 groups were analyzed using a single model. Treatment was a fixed effect and group was included as a random effect. Differences between treatments were determined using least squares means ($P < 0.05$).

Phase 4: Monitoring for PCV2 under commercial conditions

A commercial farm in Kansas, determined to have had severe PCVD before circovirus vaccine became available, was selected as a herd for an additional monitoring study (Phase 4) because of proximity and clinical history. Prior to the introduction of circovirus vaccine, postweaning mortality had ranged from 5% to 19%. After implementation of a circovirus vaccination program (Circumvent PCV) the herd had less apparent clinical disease (mortality: 4% to 9%). The circovirus vaccination program had been in place for a year before our Phase 4 study began. In addition to the history of PCV2 infection, porcine reproductive and respiratory syndrome virus and *M. hyo* also contributed to the health challenges in the nursery and finishing phases of production. Pigs were weaned from a sow farm in western Kansas and moved to eastern Kansas to be placed at a nursery-finishing site. There were 2 nursery barns with 4 rooms each and 8 finishing barns at the site. Pigs were moved all-in, all-out by nursery room and finishing barn.

A total of 85 pigs (1.7 to 3.1 wk of age) from a 1,100 pig weaning group were ear-tagged and bled just prior to weaning. These 85 pigs were not vaccinated against PCV2 and were monitored for 9 wk. All other pigs in the weaning group were vaccinated according to standard farm protocol with a 2-dose circovirus vaccine (Circumvent PCV). The 85 non-vaccinated sentinel pigs were initially penned in 4 pens in the nursery room which also contained pens of circovirus-vaccinated pigs. If pigs were removed from their initial pens because of illness or injury, they were moved to a sick pig pen but were still monitored. After approximately 8 weeks in the nursery, pigs were moved to a single finisher barn at the same farm location and were placed in pens according to their vaccination status. Pigs were bled approximately every 3 wk for a total of 4 sampling times (sampling time age ranges: 1.7 to 3.1, 4.9 to 6.3, 7.9 to 9.3, 10.9 to 12.3 wk of age). The objective of this monitoring effort was to determine whether non-

vaccinated pigs housed in barns with pigs vaccinated against PCV2 became viremic with PCV2 after circovirus vaccine was used in the herd for a year.

Serum samples were pooled (5 samples per pool) within age range and were analyzed by the KSVDL PCV2 PCR assay for presence of PCV2 nucleic acid. Genotype of PCV2 (PCV2a or PCV2b) was determined for samples with detectable PCV2 nucleic acid.

Phase 5: Monitoring for PCV2 in the environment of swine barns

As pigs involved in all previous phases of this study were exposed to different environments and pigs over time, it was of interest to determine whether there were documentable sources of PCV2 exposure. The objective for this phase of monitoring was to demonstrate applicability of swabbing and PCV2 PCR testing as a method for monitoring PCV2 levels on environmental surfaces in swine production facilities.

Swab samples were collected from the nursery and finisher rooms at both the KSU farm and the commercial farm in eastern Kansas which was used in the Phase 4 study. Cotton swabs were used to sample the floor slats, gating, waterers, feeders, fans and heaters in the nursery or finishing rooms. Swabs were placed in vials containing enriched media. For each farm, samples were pooled within nursery or finishing production phases (2 KSU nursery or finishing pools and 16 commercial farm nursery or finishing pools). A uniform amount of this pooled suspension was tested by KSVDL PCV2 PCR for detection of PCV2 nucleic acid.

Results

Phase 1:

Baseline PCV2 IFA testing of the serum collected from pigs from the MSU herd demonstrated that passively-acquired antibody declined by 15 wk of age (Figure 5.1). Higher levels of antibody were apparent in pigs 16 to 20 wk of age or older. There was PCV2 nucleic acid detected by PCR in serum samples from pigs 11 to 15 wk of age and older (Figure 5.2).

In the baseline analysis of the KSU herd (Phase 1), passively-acquired antibody in growing pigs declined by 19 wk of age with higher levels of antibody detected following this decline (Figure 5.3). Viremia was detectable only in populations consisting of pigs which were 19 and 24 wk of age (Figure 5.4). The 19-wk old pigs were viremic but did not have antibody levels suggestive of seroconversion.

Phase 2:

After introduction of circovirus vaccination, PCV2 PCR testing of serum samples collected over time from 9 MSU and 10 KSU cohort groups showed a different infection pattern on each farm compared with baseline PCR profiles. From the MSU farm, PCV2 PCR testing on sera collected from 86 barrows at 4 sampling points (pre-vaccination, entry-to-finishing, mid-finishing, and end-of-finishing) failed to detect PCV2 nucleic DNA (Table 5.1).

From the KSU farm, testing by PCV2 PCR on serum samples from 111 barrows failed to detect nucleic acid (PCV2 PCR negative) in samples collected at any time from pigs in groups 1, 2, 4, 7, 8, 9, and 10 (Table 5.2). Serum samples with detectable PCV2 DNA (PCV2 PCR positive) were found in group 3 (10%, 1/10 samples from mid-finishing), group 5 (25%, 3/12 samples from weaning; 25%, 3/12 samples from entry-to-finishing; 8.3%, 1/12 samples from mid-finishing; and 8.3%, 1/12 samples from end-of-finishing), and group 6 (8.3%, 1/12 samples from entry-to-finishing). For serum samples with detectable DNA, viral template quantity ranged from 5 to 379 viral template copies per reaction. In only 1 (group 5) of the 10 groups (10%) did a pig remain viremic for longer than 1 testing interval. Overall, no PCV2 viral DNA was detected in samples from 7 of the 10 groups (70%) monitored over a time period of greater than 1 year.

Phase 3:

After 2 years of vaccinating growing pigs against PCV2 at the KSU farm, subsamples of pigs were allocated to a circovirus-vaccinated treatment or a non-vaccinated control treatment in a growth and PCV2 antibody follow-up study (Phase 3). There was an interaction ($P < 0.001$) between treatment and time for antibody level (Table 5.3). With the exception of the initial bleed (d 0; during the wk of weaning) when control and vaccinated pig antibody levels were similar ($P = 0.41$), vaccinated pigs had increased ($P < 0.001$) PCV2 antibody levels compared with controls at all other sampling times. The magnitude of the antibody responses varied over time for control and vaccinated pigs as did the pattern of antibody production or decay. By the time the pigs were placed into the finisher, control pig antibody levels had declined ($P < 0.001$) compared with their respective d 0 levels; however, throughout the finishing period control pig antibody levels remained similar ($P \geq 0.61$). In contrast, compared with their respective d 0 antibody levels, vaccinated pigs had an increase ($P < 0.001$) in PCV2 antibody titer by the time of entering the finisher which decreased ($P < 0.001$) by each of the subsequent sampling points.

During the nursery and grower periods, vaccinated pigs had decreased ($P = 0.005$; Table 5.4) ADG compared with non-vaccinated control pigs. Vaccinated and control pigs had similar ($P = 0.30$) finishing ADG though growth rates for vaccinated pigs continued to be numerically less than control pig growth rates. Overall, there was a tendency ($P = 0.07$) for vaccinated pigs to have decreased ADG compared with control pigs. These growth rate differences resulted in control pigs entering the finisher being 1.2 kg heavier ($P = 0.03$) than vaccinated pigs. When pigs were taken off test at the end of the finishing period, control pigs had a numeric weight advantage ($P = 0.16$) of 2.0 kg over vaccinated pigs.

Phase 4:

Results obtained from the commercial farm with a 1-year history of circovirus-vaccination were different than those observed in the KSU farm. From a serial sampling of 85 non-vaccinated sentinel pigs, there was no PCV2 DNA detected in the weaning pools (0/17 pools; Table 5.5). In contrast, PCV2 nucleic acid was detected in pooled samples at each 3 subsequent sampling ages (4.9 to 6.3 wk of age: 1/17 pools; 7.9 to 9.3 wk of age: 6/16 pools; and 10.9 to 12.3 wk of age: 12/16 pools). Genotype was reported for each pool. There was PCV2a detected in all but 1 pool (4.9 to 6.3 wk of age: 1/17 pools; 7.9 to 9.3 wk of age: 6/16 pools; and 10.9 to 12.3 wk of age: 11/16 pools). However, PCV2b was not detected in any of the pools until 10.9 to 12.3 wk of age (2/16 pools).

Phase 5:

Environmental swabbing and testing by PCV2 PCR (Figure 5.5) detected PCV2 DNA in samples from 8 commercial nursery and 8 commercial finisher barns. In contrast, the presence of PCV2 DNA was not detected by PCV2 PCR testing of environmental swab samples from the KSU farm.

Discussion

Porcine circovirus disease is considered to be a leading viral disease in the swine industry (Opriessnig et al., 2009). Circovirus vaccines are available as an aid for prevention of viremia but these vaccines are costly to production systems. In addition, concern that the field viral strains may mutate thereby reducing the effectiveness of vaccines demands consideration of other options such as PCV2 elimination.

This was a first study to evaluate the effects of circovirus vaccination on viral circulation at the herd level. Determining whether vaccination could be used as a tool in viral elimination efforts was a focus for our study because it was known that vaccination against PCV2 reduced viremia (Fachinger et al., 2008) and viral shedding (Fort et al., 2009). Our study was designed to begin to evaluate the hypothesis that circovirus vaccination programs in herds would affect viremia and subsequent viral shedding into the environment. Over time, a reduction in environmental contamination coupled with continued use of circovirus vaccine to build immunity in growing pigs prior to viral exposure, would aid derivation of PCV2-free herds.

The MSU and KSU herds and management served as models for commercial multi-site swine production systems. Based on the Phase 1 baseline testing, PCV2 was detected in both swine populations though viremia was not increased until after the nursery period. This provided evidence for primarily horizontal rather than vertical transmission. Both herds had PCV2-viremic pigs during finishing and had evidence that pigs likely seroconverted after the documented time for onset of viremia (Figures 5.1, 5.2, 5.3, and 5.4).

Although both farms had viral circulation evident during finishing, the MSU pigs experienced an earlier onset of viremia than the KSU pigs. Both herds were considered good models in which to monitor the effects of circovirus vaccination long-term because baseline results from both non-vaccinated populations indicated viral presence and seroconversion-supporting antibody profiles.

Circovirus vaccination programs were started in each herd in the spring of 2007 and monitoring of barrows from each farrowing group began. Barrows were selected for monitoring because some reports have indicated that barrows were at increased risk for development of disease (Corrége et al., 2001; Rodríguez-Arriola et al., 2002). In the MSU herd, viremia was not detected in serum collected at any sampling point from circovirus-vaccinated barrows (Table 5.1). During the same time, there were no reports of clinical PCVD from the farm. Still, it is possible that some pigs may have become transiently viremic between sampling points. In a study under field conditions, compared with non-vaccinated control pigs, circovirus vaccination reduced ($P < 0.001$) average duration of viremia by almost 50% (non-vaccinated control pigs: 34.3 d vs. vaccinated pigs: 17.4 d) (Fachinger et al., 2008). However, the MSU farm baseline testing indicated onset of viremia early in the finishing phase and infection appeared to be detectable in a portion of the population throughout finishing. Thus, the MSU vaccinated pig

PCR data demonstrates that vaccination appeared to have an effect on the viral circulation within this farm by either shortening the duration of viremia or preventing it altogether.

In the KSU herd, there were 3 groups which had at least 1 pig with detectable PCV2 DNA in the serum. These groups (3, 5, and 6; Table 5.2) were not consecutive groups, nor were the ages at the time of detectable viremia consistent among groups. In addition, there was only 1 group with pigs testing positive for PCV2 at more than 1 sampling point. Although the viral load levels between sampling points were not known, the PCV2 viral loads detected in the positive serum samples among the 4 bleeding times were 379 template copies per reaction or less. The biologic significance of these numbers is not yet known: however, viral burden has been positively correlated with histologic lesion severity in non-vaccinated and PCV2-challenged pigs (Krakowka et al., 2005). In our study, none of the viremic vaccinated pigs or their group-mates had been identified as being PCVD-suspects. Evidence of PCV2-problems was restricted to PCR detection of transient viremia. Though PCV2 was intermittently detected among vaccinated pigs, because there were no naïve pigs in the population the virus was not able to transmit readily, propagate within groups, and establish widespread infection within the herd. Therefore, these KSU herd results indicate immunization by circovirus vaccination affected viral circulation by controlling the spread of virus and shortening the duration of viremia or by preventing the infection entirely.

The follow-up study (Phase 3) was performed at the KSU farm to verify circovirus vaccination had affected within-in farm viral circulation patterns and to determine the farm's new PCV2-status. Results indicate a change in the herd PCV2 antibody profile. Pigs for this follow-up study were born primarily from dams that were vaccinated against circovirus as weaned pigs; however, gilts or sows were not vaccinated against circovirus prior to breeding or during gestation. Whereas before vaccine introduction into the herd, pigs had antibody decay until mid-finishing followed by high levels of antibody in late-finishing, the pattern after 2 years of continuous vaccination was different. Antibody levels at the time of weaning were similar and low for pigs assigned to the control or vaccinated treatments (Table 5.3). After vaccination, vaccinated pigs had a rise in antibody by the beginning of the finishing period which then decreased throughout finishing. In contrast, control pigs had decay in antibody levels through the beginning of finishing and never had a rise in antibody levels. The lack of antibody rise suggests that control pigs were not exposed to the PCV2 virus during the time period for

sampling. Residual PCV2 virus shed from previous infected pigs and present in the environment did not appear to stimulate an immune response in these control pigs, nor did it appear that there was exposure to PCV2 virus transmitted from vaccinated but infected pigs within the groups. These follow-up KSU results indicate that the virus had either been eliminated from the herd and farm facilities, or had fallen below a threshold which could trigger stimulation of the immune system.

Growth rate had been previously used as an indicator of disease and therefore was included as a response for this study. Previous field studies with natural PCV2-challenge indicated that pigs vaccinated against PCV2 had improved overall growth rates compared with non-vaccinated control pigs with the largest improvements in ADG detected during finishing (Fachinger et al., 2008; Horlen et al., 2008; Jacela et al., 2011). Although usually beneficial for finishing performance, some studies have demonstrated negative effects on nursery pig growth performance with vaccination programs involving the 2-dose circovirus vaccine (Potter et al., 2009; Shelton et al., 2009; Vilaca et al., 2010).

In our study, circovirus vaccination negatively affected growth rate during the nursery and grower periods (Table 5.4). This resulted in vaccinated pigs being 1.2 kg lighter than non-vaccinated control pigs at the beginning of the finishing period. These results are consistent with other work which indicated that pigs vaccinated with the 2-dose circovirus vaccine (Circumvent PCV) have reduced growth rates after vaccination compared with either non-vaccinated control pigs or pigs vaccinated with the 1-dose circovirus vaccine (CircoFLEX; Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) (Potter et al., 2009).

During the finisher phase and for the overall study, vaccinated pigs had numerically reduced ADG compared with control pigs. At the time pigs were taken off test, compared with vaccinated pigs, control pigs had a 2.0 kg numeric weight advantage. However, the lack of positive growth rate response due to vaccination may be explainable by low or no natural PCV2 challenge in the KSU herd. We believe that the level of PCV2 viral exposure had been reduced or eliminated on this farm due to the circovirus vaccination program and the management practices used during the previous 2 years. However, other researchers have found that under conditions where there was a natural PCV2 challenge, vaccination improved finishing pig performance compared with that of non-vaccinates (Horlen et al., 2008; Jacela et al., 2011).

In our study, during finishing, vaccinated pigs did not demonstrate greater ADG compared with non-vaccinated control pigs. Vaccinated pigs were not able to compensate for or overcome the negative effects of vaccination in the nursery. Thus, the immunity built in the nursery and grower period did not provide any benefit during finishing as PCV2 was not present as a challenge to the immune system of the pigs. Therefore, the lack of serologic evidence for PCV2 exposure coupled with the tendency for vaccinated pigs to have poorer overall growth performance than control pigs suggests that PCV2 was not a pathogenic threat for growing pigs in the KSU herd during the follow-up testing.

The results which indicated that PCV2 was no longer an apparent natural challenge for pigs in the KSU farm could not be replicated in a commercial farm in Kansas despite both farms having implemented long-term circovirus vaccination programs. At the time the data were collected, the commercial farm had been continuously vaccinating pigs for a 1 year—slightly less time than the KSU farm. Clinical disease had been decreased during the time when the vaccine was being used in the commercial herd. The commercial farm moved pigs all-in, all-out from their nursery and finisher rooms and used a similar disinfectant to that of the KSU farm. However, the period of down-time between batches of pigs for cleaning and disinfection of rooms was longer at the KSU farm compared to the commercial farm.

In the commercial farm, the non-vaccinated pigs did become viremic after movement into the nursery (Table 5.5) and exhibited clinical signs of PCVD. The clinical disease in these pigs was apparent even though they constituted a relatively low percentage of the population and herd immunity did not appear to prevent propagation of the infection. Therefore, the belief that housing environment contributed a significant source of PCV2 virus in this population led us to perform the environmental evaluation. We acknowledge that pig-to-pig transmission from viremic pigs could also play a role in the dynamics of the infection. However, we believe this was less likely. At each time point, more serum pools had detectable DNA, which indicated that more pigs were becoming infected. In addition, PCV2a was first detected followed by PCV2b, thus over time, the infection profile also changed. Whether this differential pattern has biologic significance is yet to be determined.

To understand why non-vaccinated pig results differed between the KSU herd and the commercial farm, it was important to identify sources of viral exposure. Seemingly pigs at both farms were being weaned free of PCV2 implicating PCV2 in the environment as a primary

source of exposure. Swabs were collected in all nursery and finishing rooms at the commercial farm. Nursery and finishing rooms at the KSU farm which had housed study pigs at some point through the 3 year study were also sampled. Though PCR detection of PCV2 nucleic acid does not provide any information about whether the viral material is infectious, it does allow measurement of environmental viral loads which could potentially contain infectious material.

In the commercial facility, PCV2 DNA was found in every room and barn. In contrast, at the KSU farm, PCV2 nucleic acid was not detected in either the nursery or finishing facility. Although the infectivity status of the PCV2 DNA detected at the commercial site was not known, any residual infectious material present in the environment could explain why non-vaccinated pigs placed in this facility became viremic shortly after movement into the facility. Complete inactivation of PCV2 was difficult by disinfection under laboratory conditions (Royer et al., 2001). Therefore, in our study, with viral material detected in the environment, it was likely that some infectious virus remained. To our knowledge, this was the first report of detection of PCV2 DNA in the environment of swine barns.

This was the also first report which has documented presence of PCV2 in the environment of a facility where non-vaccinated sentinel pigs became viremic. In contrast, at the KSU farm where presence of PCV2 was not detected in the environment, non-vaccinated pigs were not exposed to the virus. Further investigation of this environmental virus-based route of transmission is warranted to determine the importance of this potential risk.

In conclusion, results from this 3 year investigation indicate that circovirus vaccination did affect viral circulation in swine herds. Success in lowering levels or eliminating the virus as a pathogenic threat was achieved at a university research herd. However, under commercial conditions, there appeared to be other exposure risk factors, such as residual PCV2 in the environment, which inhibited viral elimination efforts. Therefore, circovirus vaccination provides a tool to affect viral circulation on farms but must be used in conjunction with other management practices to address additional exposure risk factors in order to eliminate the PCV2 virus from most swine populations.

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Figure 5.1 Characterization of the porcine circovirus type 2 (PCV2) antibody profile of the Michigan State University (MSU) Swine Teaching and Research Center herd prior to implementation of a circovirus vaccination program.

At the MSU farm, a total of 101 pigs were sampled across 5 growing pig populations (6 to 10, 11 to 15, 16 to 20, 21 to 25, and 26 to 30 wk of age) using a cross-sectional design. Serum samples from individual pigs were tested by the Kansas State Veterinary Diagnostic Laboratory PCV2 indirect fluorescent antibody (IFA) assay for detection of PCV2 antibodies. All IFA titers were \log_2 transformed to approximate a normal distribution prior to descriptive analysis. Resulting transformed means were transformed back to the original scale for presentation as geometric mean titers (GMT).

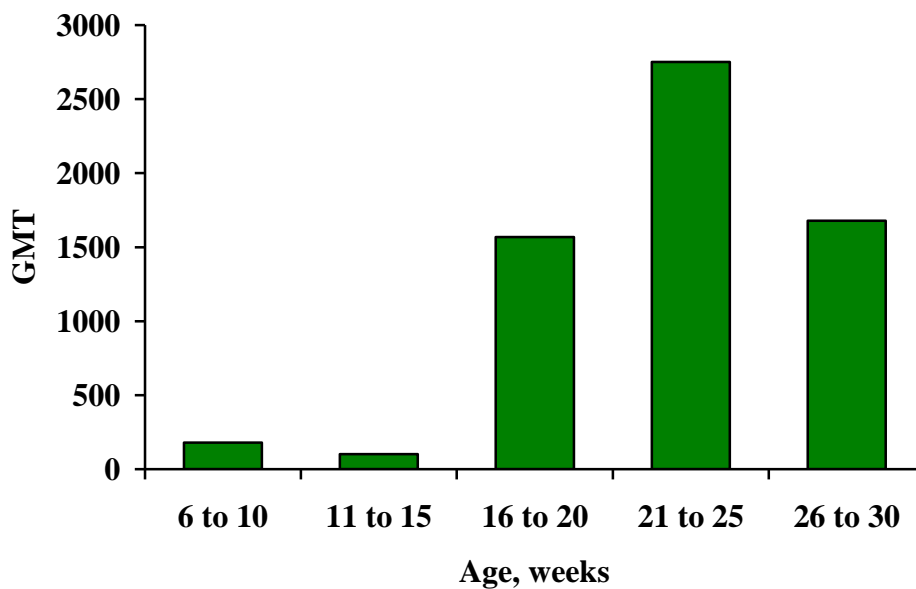


Figure 5.2 Characterization of the porcine circovirus type 2 (PCV2) infection profile of the Michigan State University (MSU) Swine Teaching and Research Center herd prior to implementation of a circovirus vaccination program.

Serum was pooled (MSU: 21 pools) within age group and analyzed using the Kansas State Veterinary Diagnostic Laboratory (KSVDL) PCV2 polymerase chain reaction assay for detection of PCV2 nucleic acid. Pooled results were \log_{10} transformed and transformed results were averaged within age ranges to characterize patterns for viral load.

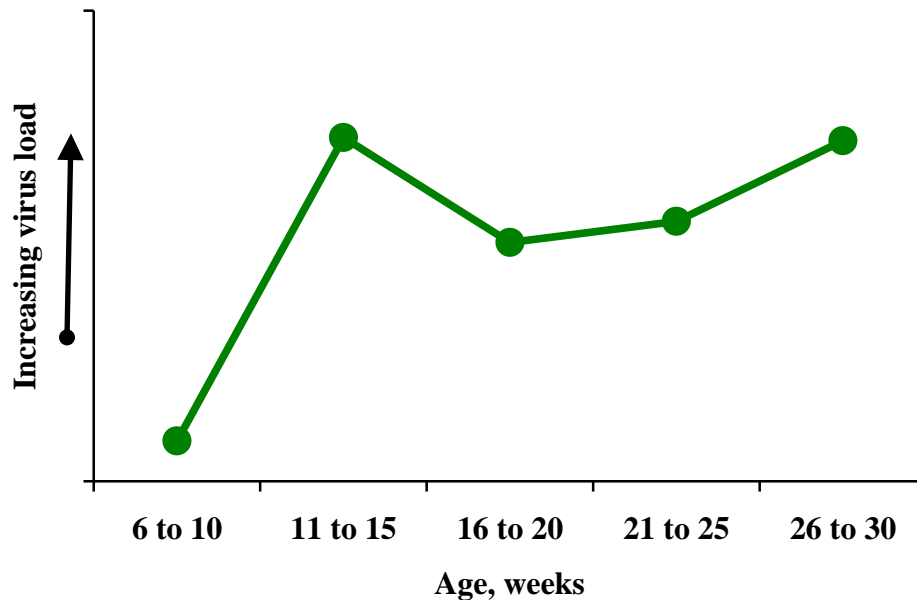


Figure 5.3 Characterization of the porcine circovirus type 2 (PCV2) antibody profile of the Kansas State University (KSU) Swine Teaching and Research Center herd prior to implementation of a circovirus vaccination program.

At the KSU farm, a total of 141 pigs were sampled across 5 growing pig populations (4, 9, 14, 19, and 24 wk of age) using a cross-sectional design. Serum samples from individual pigs were tested by the Kansas State Veterinary Diagnostic Laboratory PCV2 indirect fluorescent antibody (IFA) assay for detection of PCV2 antibodies. All IFA titers were \log_2 transformed to approximate a normal distribution prior to descriptive analysis. Resulting transformed means were transformed back to the original scale for presentation as geometric mean titers (GMT).

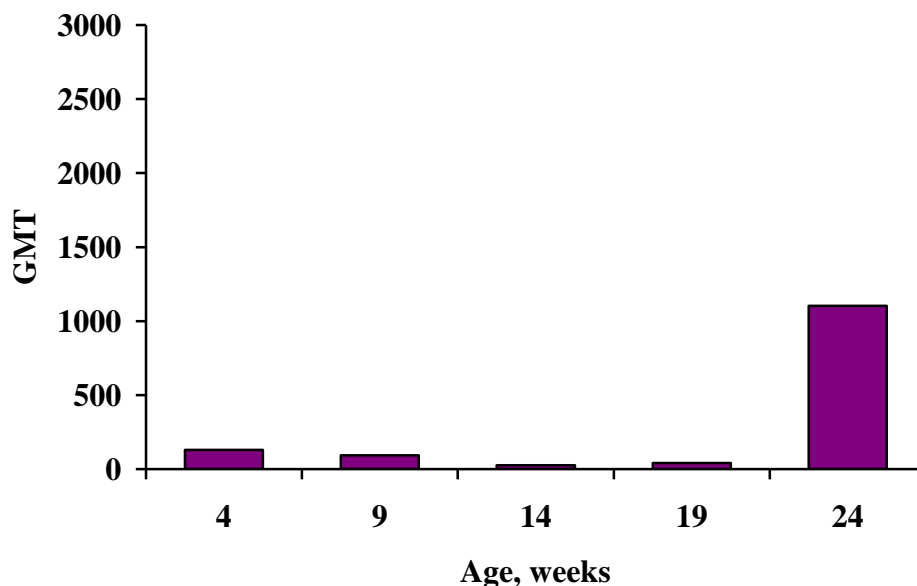


Figure 5.4 Characterization of the porcine circovirus type 2 (PCV2) infection profile of the Kansas State University (KSU) Swine Teaching and Research Center herd prior to implementation of a circovirus vaccination program.

Serum was pooled (KSU: 27 pools) within age group and analyzed using the Kansas State Veterinary Diagnostic Laboratory PCV2 polymerase chain reaction assay for detection of PCV2 nucleic acid. Pooled results were \log_{10} transformed and transformed results were averaged within age ranges to characterize patterns for viral load.

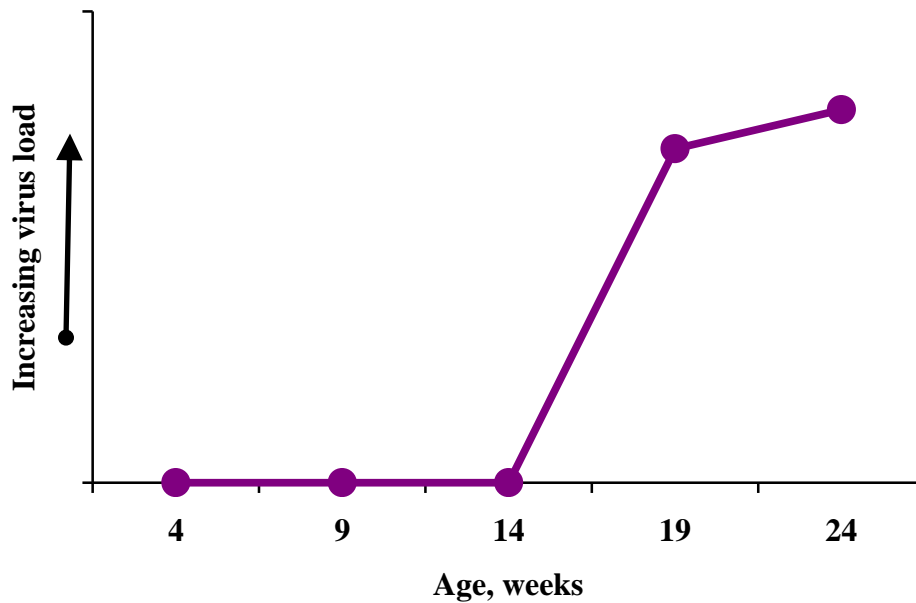
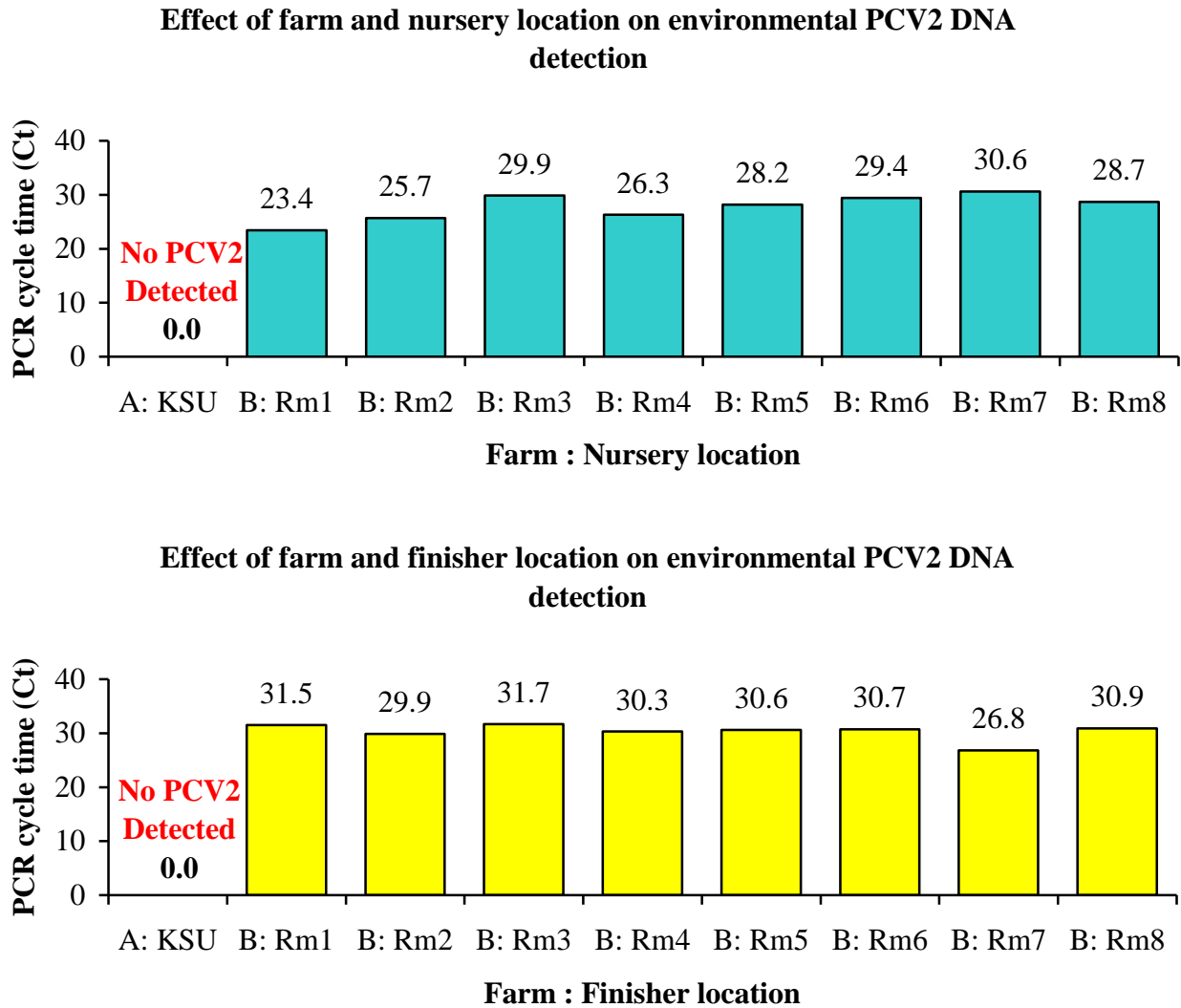


Figure 5.5 Detection of porcine circovirus type 2 (PCV2) nucleic acid in the environment of nursery and finisher facilities at the Kansas State University (KSU) Swine Teaching and Research Center and a commercial farm.



Note. Porcine circovirus type 2 (PCV2) polymerase chain reaction (PCR) results for environmental swabs of Farm A (KSU farm) and Farm B (commercial farm) nursery and finisher locations. Cycle time (Ct) values are reported as 0.0 (no PCV2 DNA detected) or greater than 0.0 (PCV2 DNA detected) with the lower positive Ct values indicative of more PCV2 viral DNA.

Table 5.1 Detection of porcine circovirus type 2 (PCV2) nucleic acid in serum samples serially-collected from barrows across 9 consecutive weaning groups enrolled in a post-circovirus-vaccination implementation monitoring program at the Michigan State University Swine Teaching and Research Center¹

Item	Pigs, no. ³	Sampling ²			
		d 0 (wean wk)	Entry-to- finishing	Mid- finishing	End-of- finishing
Group 1	10				
Interval, wk ⁴		---	6.1	14.0	17.1
PCV2 DNA detected ⁵		no	no	no	no
Group 2	9				
Interval, wk ⁴		---	7.1	15.1	18.0
PCV2 DNA detected ⁵		no	no	no	no
Group 3	9				
Interval, wk ⁴		---	5.9	12.0	18.0
PCV2 DNA detected ⁵		no	no	no	no
Group 4	9				
Interval, wk ⁴		---	7.0	13.0	18.1
PCV2 DNA detected ⁵		no	no	no	no
Group 5	11				
Interval, wk ⁴		---	5.9	12.9	16.9
PCV2 DNA detected		no	no	no	no
Group 6	10				
Interval, wk ⁴		---	6.0	11.1	18.0
PCV2 DNA detected ⁵		no	no	no	no
Group 7	10				
Interval, wk ⁴		---	8.1	15.0	20.0
PCV2 DNA detected ⁵		no	no	no	no
Group 8	9				
Interval, wk ⁴		---	6.3	13.1	18.0
PCV2 DNA detected ⁵		no	no	no	no
Group 9	9				
Interval, wk ⁴		---	6.0	13.0	18.0
PCV2 DNA detected ⁵		no	no	no	no

¹ A total of 86 barrows (4 samples per barrow) were serially-bled and serum was analyzed by polymerase chain reaction (PCR) for detectable PCV2 DNA. All pigs were vaccinated intramuscularly with 2 doses (2 mL per dose) of Circumvent PCV (Intervet/Schering-Plough Animal Health, Millsboro, DE) after the d 0 blood sample was collected (during the wk of weaning).

² Sampling points were during wean wk (d 0; single pre-vaccination serum sample), after entry to the finisher, during mid-finishing, and at the end of the finishing period.

³ An average of 12 barrows were randomly selected across 9 consecutive farrowing groups, ear-tagged, and monitored for their lifetime. Serum samples from barrows with complete serum sets (4 serum samples per pig) only were tested by PCR for detectable PCV2 nucleic acid. Number of pigs reported in the table represents the number of pigs with complete serum sets.

⁴ Interval indicates the amount of time in wk which had elapsed since the previous sampling point. The d 0 sample was collected during weaning wk.

⁵ All serum samples were individually tested by PCR for presence of PCV2 nucleic acid. Results are reported as yes if there was a sample with detectable PCV2 nucleic acid for the indicated group and sampling point, and no if there were no samples with detectable PCV2 nucleic acid.

Table 5.2 Detection of porcine circovirus type 2 (PCV2) nucleic acid in serum samples serially-collected from barrows across 10 consecutive weaning groups enrolled in a post-circovirus-vaccination implementation monitoring program at the Kansas State University Swine Teaching and Research Center¹

Item	Pigs, no. ³	Sampling ²			
		d 0 (wean wk)	Entry-to- finishing	Mid- finishing	End-of- finishing
Group 1	11				
Interval, wk ⁴		---	8.7	15.0	21.9
PCV2 DNA detected ⁵		no	no	no	no
Group 2	10				
Interval, wk ⁴		---	9.9	14.9	20.0
PCV2 DNA detected ⁵		no	no	no	no
Group 3	10				
Interval, wk ⁴		---	9.3	14.4	19.1
PCV2 DNA detected ^{5,6}		no	no	yes	no
Group 4	8				
Interval, wk ⁴		---	10.1	14.9	20.0
PCV2 DNA detected ⁵		no	no	no	no
Group 5	12				
Interval, wk ⁴		---	9.9	15.0	19.9
PCV2 DNA detected ^{5,6}		yes	yes	yes	yes
Group 6	12				
Interval, wk ⁴		---	10.3	15.3	19.8
PCV2 DNA detected ^{5,6}		no	yes	no	no
Group 7	12				
Interval, wk ⁴		---	10.2	14.0	19.5
PCV2 DNA detected ⁵		no	no	no	no
Group 8	12				
Interval, wk ⁴		---	9.7	14.7	17.1
PCV2 DNA detected ⁵		no	no	no	no
Group 9	12				
Interval, wk ⁴		---	9.7	15.0	19.6
PCV2 DNA detected ⁵		no	no	no	no

Group 10	12			
Interval, wk ⁴	---	10.3	14.9	20.3
PCV2 DNA detected ⁵	no	no	no	no

¹ A total of 111 barrows (4 samples per barrow) were serially-bled and serum was analyzed by polymerase chain reaction (PCR) for detectable PCV2 DNA. All pigs were vaccinated intramuscularly with 2 doses (2 mL per dose) of Circumvent PCV (Intervet/Schering-Plough Animal Health, Millsboro, DE) after the d 0 blood sample was collected (during the wk of weaning).

² Sampling points were during wean wk (d 0; single pre-vaccination serum sample), after entry to the finisher, during mid-finishing, and at the end of the finishing period.

³ An average of 12 barrows were randomly selected across 10 consecutive farrowing groups, ear-tagged, and monitored for their lifetime. Serum samples from barrows with complete serum sets (4 serum samples per pig) only were tested by PCR for detectable PCV2 nucleic acid. Number of pigs reported in the table represents the number of pigs with complete serum sets.

⁴ Interval indicates the amount of time in wk which had elapsed since d 0 (day of vaccination). The d 0 sample was collected during weaning wk and was collected before the vaccine was administered.

⁵ All serum samples were individually tested by PCR for presence of PCV2 nucleic acid. Results are reported as yes if there was a sample with detectable PCV2 nucleic acid for the indicated group and sampling point, and no if there were no samples with detectable PCV2 nucleic acid.

⁶ Viral template quantities ranged from 5 to 379 template copies per reaction across serum samples with detectable PCV2 nucleic acid. Within group 5 pigs, there were 2 barrows with serum samples which had detectable nucleic acid at more than 1 sampling point.

Table 5.3 Effect of circovirus vaccination and time on indirect fluorescent antibody (IFA) geometric mean titer (GMT) in pigs produced at a farm which had been vaccinating growing pigs against porcine circovirus type 2 (PCV2) continuously for 2 years¹

		Treatment ²								Probability, <i>P</i> <
		Control				Vaccinate				
		d 0 (wean wk)	Entry-to- finishing	Mid- finishing	End-of- finishing	d 0 (wean wk)	Entry-to- finishing	Mid- finishing	End-of- finishing	
Item	Time:									
Samples, no.		68	68	68	68	68	66	66	66	---
Titer, GMT ³		35.9 ^a	15.2 ^b	14.8 ^b	13.6 ^b	43.6 ^a	52789.3 ^c	13841.2 ^d	3729.8 ^e	<0.001

¹ A total of 136 barrows (68 control and 68 vaccinated pigs) across 3 farrowing groups were ear-tagged and monitored from weaning through finishing at the Kansas State University Swine Teaching and Research Center. Pigs were serially-bled on d 0 (within a wk of weaning), after entering the finisher (time elapsed since d 0 range: 8.4 to 8.9 wk), mid-finishing (time elapsed since d 0 range: 13.4 to 13.9 wk), and at the end of the finishing period (time elapsed since d 0 range: 18.0 to 19.4 wk). Antibody levels against PCV2 were determined by IFA testing on individual serum samples. Individual pig IFA titer data were log₃ transformed and were analyzed by repeated measures analysis using the GLIMMIX procedure in SAS version 9.1.3 (SAS Institute, Inc., Cary, NC). Fixed effects in the model included treatment, time, and their interaction. Group and IFA day were included as random effects.

² Treatments were non-vaccinated control or vaccinated. Vaccinated pigs were injected intramuscularly with 2 doses (2 mL per dose) of Circumvent PCV (Intervet/Schering-Plough Animal Health, Millsboro, DE) after the d 0 blood sample was collected (during the wk of weaning).

³ Geometric mean titers were calculated by taking the mean of the log₃ transformed IFA titer values then converting the resulting transformed mean back to the original scale for presentation.

^{a,b,c,d,e} Means without a common superscript letter differ ($P < 0.05$).

Table 5.4 Effect of circovirus vaccination on growth rate of pigs produced at a farm which had been vaccinating growing pigs against porcine circovirus type 2 (PCV2) continuously for 2 years¹

Item	Treatment ²		SEM	Probability, <i>P</i> <
	Control	Vaccinate		
Pigs started on test, no.	186	186	---	---
ADG, g				
Nursery-grower ³	561	537	6.1	0.005
Finisher ⁴	1,095	1,083	13.1	0.30
Overall ⁵	853	838	10.6	0.07
Weight, kg				
d 0	6.3	6.3	0.20	0.97
Entry-to-finishing	40.4	39.2	0.90	0.03
End-of-finishing (off test)	119.2	117.2	2.42	0.16

¹ A total of 372 weanling pigs (186 control and 186 vaccinated pigs) across 3 farrowing groups were ear-tagged and monitored from weaning through finishing at the Kansas State University Swine Teaching and Research Center. Pigs were individually weighed on d 0 (within the weaning wk and the day of vaccination), after entering the finisher, and at the end of the finishing period to calculate average daily gain. Growth and on test time data from mortalities and removed pigs were included in growth and period length calculations. Individual pig growth data were analyzed using the GLIMMIX procedure in SAS version 9.1.3 (SAS Institute, Inc., Cary, NC). The interaction with gender and treatment was determined to be non-significant for group 3, and growth data was pooled across the genders for subsequent analysis. Growth data for all 3 groups was analyzed using a model which included treatment as a fixed effect and group as a random effect.

² Treatments were non-vaccinated control or vaccinated. Vaccinated pigs were injected intramuscularly with 2 doses (2 mL per dose) of Circumvent PCV (Intervet/Schering-Plough Animal Health, Millsboro, DE).

³ Nursery-grower ADG and period length include data from mortalities and removed pigs. The nursery period length was not different ($P = 0.15$) between control (59.7 ± 1.48 d) and vaccinated (59.1 ± 1.48 d) pigs.

⁴ Finisher ADG and length include data from mortalities and removed pigs. The number of days for the finisher period was not different ($P = 0.94$) between control (71.7 ± 1.13 d) and vaccinated (71.6 ± 1.14 d) pigs.

⁵ Overall ADG and length include data from mortalities and removed pigs. The number of days for the overall trial was not different ($P = 0.96$) between control (132.1 ± 2.67 d) and vaccinated (132.1 ± 2.67 d) pigs.

Table 5.5 Detection of porcine circovirus type 2 (PCV2) nucleic acid in serum samples serially-collected from pigs not vaccinated for PCV2 in a monitoring program at a commercial farm¹

Item	Age, wk ²			
	1.7 to 3.1 (wean wk)	4.9 to 6.3	7.9 to 9.3	10.9 to 12.3
Pig survival, %	100.0	97.6	91.8	91.8
Interval, wk ³	---	3.2	6.2	9.2
PCV2 PCR results				
Pools for PCR, no. ⁴	17	17	16	16
PCV2 DNA detected ^{5,6}	no	yes	yes	yes
Pools with detectable PCV2 DNA, %	0	5.9	37.5	75.0

¹ A total of 85 pigs were serially-bled and serum was analyzed by polymerase chain reaction (PCR) for detectable PCV2 DNA. Pigs were not vaccinated for PCV2 at any time during this monitoring period on this commercial farm.

² Pigs were bled initially during the wk of weaning when pig ages ranged from 1.7 to 3.1 wk of age. Pigs were serially-bled every 3 wk (on average) thereafter until pigs were 10.9 to 12.3 wk of age.

³ Interval indicates the amount of time in wk which had elapsed since the initial sampling point. The initial sample was collected during weaning wk.

⁴ A total of 5 serum samples were included in a single pool for testing by PCV2 PCR.

⁵ All serum samples were individually tested by PCR for presence of PCV2 nucleic acid. Results are reported as yes if there was a sample with detectable PCV2 nucleic acid for the indicated group and sampling point, and no if there were no samples with detectable PCV2 nucleic acid.

⁶ For serum pools with PCV2 DNA detected, cycle time (Ct) values ranged from 27.7 to 40.7.

CHAPTER 6 - Effect of vaccination with a subunit vaccine for porcine reproductive and respiratory syndrome virus on mortality and finishing pig performance under field conditions

Summary

Objective(s): The objectives of this trial were to evaluate the effect of vaccination with a subunit porcine reproductive and respiratory syndrome virus vaccine (PRRSV-RS; Sirrah Bios, Ames, IA) on cumulative mortality and finishing performance of pigs under field conditions.

Materials and methods: A total of 140 litters of pigs (1,561 pigs) were allotted to different vaccination treatments (vaccinated or non-vaccinated control) at the time of processing (4 d of age). Treatment groups were formed by randomly assigning one of the vaccination treatments to the first litter processed at each of 5 sow farms. Vaccination treatment assignments were then alternated on subsequently processed litters. The non-vaccinated control group consisted of 780 pigs, from a total of 70 litters, while the vaccinated group included a total of 781 pigs, also representing 70 litters. Pigs assigned to the vaccinated treatment were injected with 1 mL PRRSV-RS vaccine intramuscularly at processing (4 d of age) and again at weaning (approximately 24 d of age). Mortality was recorded from the day of processing through finishing. Pigs were weaned into a common nursery and control and vaccinated pigs were comingled within pens. Upon movement to the finishing barn, pigs were penned by gender and vaccine status (25 control pig pens; 24 vaccinated pig pens). Pigs were first weighed 2 wk after placement into the finishing barn (d 0) and approximately every two weeks until d 112 (off-test). On d 90, 168 heavy pigs (84 control pigs and 84 vaccinated pigs) were weighed, taken off test, and sold as top pigs. Mortality data were analyzed using chi-square analysis while growth data were analyzed by analysis of variance using a mixed model.

Results: Cumulative mortality risks (processing through nursery and overall) were not affected ($P \geq 0.23$) by vaccination treatment. There were no 2-way interactions ($P \geq 0.10$) between gender and vaccination treatment for any d 0 to 112 performance responses. Two weeks into the

finishing period (d 0), mean weights of control (26.5 kg) and vaccinated pigs (26.6 kg) were not different ($P = 0.90$). Control and vaccinated pigs had similar ($P \geq 0.13$) mean ADG, ADFI, and G:F throughout the finishing period. Average off-test mean BW of control and vaccinated pigs were not different ($P = 0.95$).

Implications: The subunit PRRSv vaccine (PRRSV-RS) used in this study failed to affect overall mortality or finishing growth performance of commercial pigs.

Key words: growth, mortality, pig, PRRSv, vaccine

Porcine reproductive and respiratory syndrome has been recognized in the United States since 1987 (Christianson and Joo, 1994). This economically-destructive disease is caused by porcine reproductive and respiratory syndrome virus (PRRSv) a member of the family *Arteriviridae*. Modified-live and whole virus inactivated PRRSv vaccine products have been produced for commercial use (Hill et al., 2004); however, success with these vaccines to control or prevent PRRSv infection has been limited. Modified live vaccines tended to reduce lesions and clinical signs when used with a homologous virus strain; however, with heterologous PRRSv strains the vaccines were less effective (Labarque et al., 2003). In general, inactivated PRRSv vaccines work poorly in naïve animals; yet, they may have a functional role in boosting antibody titers (Hill et al., 2004). Porcine reproductive and respiratory syndrome virus strains can mutate (Chang et al., 2002) which, combined with the capability of the virus to evade some immune functions (Pol and Steverink, 2000), has challenged vaccine development. To date a consistently effective vaccine has not been identified. In addition, the attenuated vaccine virus from modified-live PRRSv vaccines is shed after vaccine administration and can transmit to unvaccinated pigs (Botner et al., 1997; Nielsen et al., 2001). With vaccine virus being shed, there is concern that further transmission of the PRRSv vaccine virus may increase the potential for reversion to a more virulent viral form (Opriessnig et al., 2002).

Subunit vaccines, a class of second generation vaccines which are developed using specific viral proteins, have been effective for prevention of other diseases such as porcine circovirus disease (Fachinger et al., 2008; Horlen et al., 2008). Subunit products have some properties desirable in a vaccine. Made from specific proteins, no vaccine virus is shed after

administration. Subunit vaccines also allow the development of differential diagnostic tests which, based on antibody detection, characterize origins of antigen as either field virus or vaccine. In 2008, a subunit PRRSV vaccine, PRRSV-RS (Sirrah-Bios, Ames, IA), was made available in Kansas for use on sows or growing pigs. There were limited data demonstrating subunit PRRSV vaccine efficacy under field conditions. Thus, the objectives of this trial were to evaluate the effects of a subunit PRRSV vaccine (PRRSV-RS) vaccine on cumulative mortality, growth performance, and feed efficiency of commercial finisher pigs.

Materials and Methods

Procedures used in this study were approved by the Kansas State University Institutional Animal Care and Use Committee. The study was performed in a commercial multi-site production system in Kansas. Pigs were born over a 7 d period at 5 sow farms, weaned into a single nursery, and then moved to a commercial research finishing barn in northeast Kansas.

A total of 140 litters (1,561 pigs) were allotted to different vaccination treatments (vaccinated or non-vaccinated control) at the time of processing (4 d of age). Treatment groups were formed by randomly assigning the first litter processed at each of 5 sow farms to one of the vaccination treatments and then alternating treatment assignments on subsequently processed litters until each treatment was composed of pigs from 70 litters. At the time of processing, all pigs were ear-tagged with a unique colored ear-tag identifying the treatment assignment. After pigs were individually tagged, litter of origin records were not maintained. A total of 780 pigs were assigned to the non-vaccinated control treatment while 781 pigs were assigned to the vaccinated treatment. Pigs assigned to the vaccinated treatment were injected with 1 mL PRRSV vaccine (PRRSV-RS; Sirrah Bios, Ames, IA) intramuscularly at processing (4 d of age) and again at weaning (approximately 24 d of age; range: 20 to 26 d of age). Control pigs were not injected at processing or weaning.

In general, mortality was calculated by subtracting ending inventories from beginning inventories for specific periods. Mortality totals were verified by counting ear tags collected from pigs that died or were humanely euthanized in accordance with farm criteria for euthanasia. Within-period mortality was determined for the periods of processing to weaning, weaning to the end of the nursery, and throughout the finishing period until pigs were taken off test. Cumulative mortality was calculated by determining the number of pigs from each vaccination treatment

surviving at the end of the study and subtracting this value from the initial number of pigs assigned to each treatment.

Pigs from all 5 sow farms were weaned into a single nursery room and were phase-fed similar diets within phase throughout the nursery period. All pigs were allowed to have ad libitum access to feed and water throughout the trial. Pigs were penned in single-sex (barrow or gilt) pens with control and vaccinated pigs commingled within each nursery pen. All pigs were vaccinated with a 2-dose commercial circovirus vaccine (Circumvent PCV, Intervet/Schering-Plough Animal Health, Millsboro, DE) and a *Mycoplasma hyopneumoniae* vaccine (Myco Silencer ONCE, Intervet/Schering-Plough Animal Health, Millsboro, DE) during the nursery period according to routine nursery procedures.

Pigs (age range: 60 to 66 d of age) were moved to a single curtain-sided finisher barn equipped with a dual-nipple swinging drinker (Trojan Plastic Waterswing; Trojan Specialty Products, Dodge City, KS) and a single-sided, dry, 3-hole, stainless-steel feeders (AP-3WFS-QA; Automated Production Systems, Assumption, IL) in each pen. Feeder holes were 35.6 cm wide. There were 49 test pens which were 3.0 m \times 5.5 m. The barn was equipped with an automated feeding system (FeedPro; FeedLogic Corp., Willmar, MN) that recorded feed delivery to each individual pen feeder. All pigs were allowed to have ad libitum access to feed and water throughout the finishing period.

All test pigs were moved from the nursery to the finishing barn on a single day. While control and vaccinated pigs had been comingled within pens during the nursery phase, after being moved to the finishing barn, pigs were penned based on vaccination treatment (vaccinated or non-vaccinated control) and gender (barrow or gilt). Treatments were randomly assigned to pens. There were 12 pens of each treatment and gender combination with the exception of vaccinated barrows, for which there were 13 pens.

Pigs were weighed by pen and feed intake was recorded on d 0 (2 wk after placement into the finishing barn), and again on d 14, 28, 41, 56, 70, 90, and either d 111 or 112. From these data, ADG, ADFI, and G:F were calculated. According to routine farm procedures, on d 90, a total of 168 of the heaviest pigs (84 control pigs and 84 vaccinated pigs) were weighed, taken off test, and sold as top pigs. Data from these heavy pigs were included all calculations. Remaining pigs were taken off test over 2 consecutive days with the last pigs being weighed off test on d

112. A total of 24 pens (6 pens per gender and vaccination treatment) were weighed off test on the first day with the remaining pens weighed off test on the second day.

Analysis:

Mortality data were analyzed using the FREQ procedure in SAS version 9.1.3 (SAS Institute Inc., Cary, NC). Differences in mortality between treatments were determined using chi-square analysis ($P < 0.05$). Mortality data both within production period (processing to weaning, nursery, and finisher) and cumulatively were analyzed.

Finisher growth and feed performance data were analyzed as a completely randomized design using the GLIMMIX procedure of SAS version 9.1.3. Pen was the experimental unit for all performance data analysis. Vaccination treatment was the fixed effect of interest; however, gender and the interaction between vaccination treatment and gender were added to the model to control for expected differences in growth rate between barrows and gilts. Differences between treatments were determined by using least squares means ($P < 0.05$).

Results

Mortality results:

From processing (4 d of age) until weaning, there was a trend for control pigs to have increased ($P = 0.08$; Table 6.1) mortality compared with vaccinated pigs. There was no difference ($P \geq 0.20$) observed for mortality risks between control and vaccinated pigs during the nursery or finishing periods. Cumulative mortality from processing through the end of the nursery period was similar ($P = 0.23$) for controls and vaccinates. In addition, cumulative mortality from processing through off-test did not differ ($P = 0.67$) due to vaccination treatment.

Growth results:

There were no 2-way interactions ($P \geq 0.10$) observed between gender and treatment for any of the d 0 to 112 performance responses.

On d 0, the mean initial BW for barrows and gilts were similar ($P = 0.67$; Table 6.2). As expected, barrows had increased ($P < 0.001$) overall mean ADG and ADFI and poorer ($P < 0.001$) G:F than gilts. Mean off-test BW of barrows was 6.2 kg heavier ($P < 0.001$) than gilts.

Mean initial BW on d 0 were not different ($P = 0.90$; Table 6.3) for control and vaccinated pigs. Mean growth rate, ADFI, and G:F during finishing (d 0 to 112) did not differ (P

> 0.13) between vaccination treatments. There was no difference ($P = 0.95$) in mean off-test BW of control and vaccinated pigs.

Discussion

Control of PRRSv has been a challenge to the swine industry. Safe and effective vaccines which cross-protect against multiple PRRSv strains are needed to help control the problem. Commercial vaccines are available; however, to date a safe, proven vaccine which cross protects against multiple strains of PRRSv has not been identified. A PRRSv subunit vaccine (PRRSV-RS; Sirrah-Bios, Ames, IA) was recently developed for use on growing pigs or sows. The PRRSV-RS subunit vaccine has some characteristics, including differential potential, which would be appealing for use in a vaccination program focused on PRRSv elimination.

Subunit vaccines are formed by using specific viral proteins to target an immune response. Typically, these proteins are expressed using a vector that can support expression of a portion of viral immunogenic proteins (Levine and Sztein, 2004). Thus, like a whole virus inactivated vaccine product, a subunit vaccine will not propagate or revert to virulence. However, unlike a whole virus inactivated vaccine which would contain whole viral components, subunit vaccines are created from proteins of specific viral genes. Diagnostic tests to differentiate a vaccinated from a non-vaccinated but infected animal can be developed for use with subunit vaccines. Not all viral genes expressing immunogenic proteins are used in the production of a subunit vaccine, allowing a non-vaccinated but infected animal to produce an immune response against a greater number of proteins than were included in the subunit vaccine. Therefore, testing for antibodies against different vaccine and field-virus-only expressed proteins would determine whether an animal had mounted an immune response only to the vaccine or the field virus. However, an animal which was vaccinated and then exposed to a field virus would have antibodies against both antigens and not be able to be classified as vaccinated by the differential test alone.

The PRRSV-RS vaccine was developed using 2 PRRSv structural proteins in heterodimer form. This heterodimer form consists of the proteins linked by a disulfide bond (Mardassi et al., 1996). These proteins, glycoprotein 5 and the matrix protein, were those encoded by open reading frames 5, a highly variable region, and open reading frame 6, a more conserved region

among PRRSv strains (Dea et al., 2000). Both proteins were expressed using a replicon vector prior to combination with the adjuvant.

In a mouse model a heterodimer of specific proteins promoted development of neutralizing antibodies against equine arteritis virus (Balasuriya et al., 2000), also a member of the family *Arteriviridae*. Using horses in a second study, Balasuriya et al. (2002) evaluated subunit vaccines produced with a recombinant alphavirus, a vaccine strain of Venezuelan equine encephalitis, and compared responses in vaccinated horses with those from non-vaccinated horses. Vaccines used in this challenge study were derived of replicons expressing either individual equine arteritis virus proteins or the heterodimer of proteins. Horses vaccinated with the expressed heterodimer of proteins developed neutralizing antibodies, had a shorter duration shedding, and showed fewer clinical signs of disease than the other horses (Balasuriya et al., 2002). For reasons previously described, it has been suggested that the GP5-M heterodimer, a combination of proteins from both variable and conserved gene regions, may induce neutralizing antibodies against PRRSv infection in the pig which also may be cross-protective.

The herd used for this trial historically has pigs exposed to the PRRSv and clinical disease during the nursery period. In our study, during the nursery period there was no difference in mortality. It was during this period that the pigs on our study exhibited clinical signs consistent with both PRRSv and influenza. Serum was collected from different nursery pigs from the same herd during the time our trial was in progress. Diagnostic testing performed at the Kansas State Veterinary Diagnostic Laboratory confirmed presence of PRRSv by polymerase chain reaction and PRRSv antibodies by enzyme-linked immunosorbent assay. In our study, total of 9.3% of the control pigs and 9.2% of the vaccinated pigs which entered the nursery died during the nursery period. Therefore, the vaccine failed to affect on mortality during the period when control of PRRSV infection was most needed.

Once pigs were placed in the finishing barn, there was no difference in finishing mortality between control pigs (4.4%) and vaccinated pigs (5.9%). Overall, from processing through finishing, there was no difference in mortality between the vaccination treatments (control pigs: 21.5% vs. vaccinated pigs: 20.6%). Although diagnostics were not performed on trial pigs to detect viremia with PRRSv or seroconversion, pigs did exhibit clinical signs and have overall mortality consistent with other confirmed PRRSV-positive groups in this system.

Two weeks after entry into the finishing barn, there was no difference in mean pig weights between control and vaccinated pigs. Therefore, if control and vaccinated pigs weighed similarly at 4 days of age, vaccination treatment likely did not affect growth rate before the finishing period. During the 112-d finishing trial, there were no differences in ADG, ADFI, or G:F. These results indicate the PRRSV-RS vaccine did not affect performance of finishing pigs. Mean off-test weight between controls and vaccinates was similar. Thus, the PRRSV-RS vaccine failed to affect mortality, pig performance, or mean off-test weight.

Although use of the vaccine did not result in improved pig performance, it also did not negatively affect performance. Some research has indicated that a number of vaccines, with representation from subunit, inactivated, and modified-live vaccine types, can negatively impact performance after administration (Kane et al., 2009; Potter et al., 2009; Pretzer et al., 1996; Shelton et al., 2009). In a field study, nursery pig growth rate and feed intake was decreased compared with non-vaccinated controls following vaccination with a 2-dose circovirus vaccine (vaccinated on d 1 and 22 of the study). All pigs were inoculated on d 30 of the study with a live PRRRS virus just 8 d after administration of the second dose of circovirus vaccine. In the period following inoculation (d 29 to 50), feed efficiency was improved and growth rate tended to be increased in the circovirus-vaccinated pigs compared with the non-vaccinated pigs. In addition, the percentage of pigs that were able to remain on test was improved for pigs vaccinated against circovirus (Shelton et al., 2009). In our study, all pigs had been vaccinated against circovirus during the nursery period. It was not determined whether the circovirus vaccination had any effect on disease presentation during our study, though the pigs still appeared to exhibit clinical signs consistent with both PRRSV and influenza. Despite vaccination with the PRRSV-RS having no positive effects on performance or mortality percentages, there also appeared to be no additional cost associated with loss of performance with use of this vaccine. Thus, the total cost of vaccination would be due to product, labor, and supply expenses.

Therefore, in this herd, which had historical PRRSV-associated challenge, the subunit PRRSV vaccine (PRRSV-RS) failed to affect overall mortality or finishing pig performance.

Implications

- There were no differences between control and vaccinated pig mortality during any production phase or for cumulative mortality.

- Control and vaccinated pigs had similar mean ADG, ADFI, G:F, and off-test weights.
- The subunit PRRSv vaccine administered to growing pigs failed to affect mortality or finisher pig performance in this commercial herd.

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Table 6.1 Effect of PRRSV-RS vaccine on within-period and cumulative mortality^{1,2}

Item	Control	Vaccinate	Probability, <i>P</i> <
Inventory			
Processing ³	781	780	---
Weaning ⁴	707	725	---
Entry-to-finisher ⁵	641	658	---
Off-test ^{6,7}	529	535	---
Within-period mortality			
Processing to weaning, %	9.5	7.1	0.08
Nursery, %	9.3	9.2	0.95
Finisher, %	4.4	5.9	0.20
Cumulative mortality			
Processing to end-of-nursery, %	17.9	15.6	0.23
Processing to off-test, % ⁶	21.5	20.6	0.67

¹ A total of 1,561 pigs (barrows or gilts) from 140 litters across 5 sow farms were assigned to vaccination treatments at processing (4 d of age) by randomly assigning entire litters to either a vaccinated treatment or a non-vaccinated control treatment. Control and vaccinated pigs were comingled in the nursery and then separated by vaccination treatment and gender in the finishing barn. Mortality was tracked for controls and vaccinates from processing to the end of the finishing portion of the trial.

² Vaccination treatments were: Non-vaccinated (control) or vaccinated (vaccinate). Vaccinated pigs were injected intramuscularly with a 2-dose (1 mL per dose) porcine reproductive and respiratory syndrome virus subunit vaccine (PRRSV-RS; Sirrah-Bios, Ames, IA) at processing and weaning.

³ 4 d of age.

⁴ Weaning age range was 20 to 26 d of age.

⁵ Entry-to-finisher age range was 60 to 66 d of age.

⁶ Off-test age range was 187 to 193 d of age.

⁷ Inventory at off-test excludes pigs sold as top pigs (84 controls and 84 vaccinates) on d 90 of the trial.

Table 6.2 Means and standard errors for growth performance responses for barrows and gilts¹

Item	Barrow	Gilt	Probability, <i>P</i> <
Initial BW on d 0, kg	26.3 ± 0.77	26.8 ± 0.79	0.67
d 0 to 112			
ADG, kg	0.911 ± 0.0119	0.850 ± 0.0121	< 0.001
ADFI, kg	2.561 ± 0.0361	2.297 ± 0.0368	< 0.001
G:F	0.356 ± 0.0026	0.370 ± 0.0027	< 0.001
Mean off-test BW, kg	129.9 ± 1.06	123.7 ± 1.08	< 0.001

Note. Results are reported as least squares mean ± standard error of the mean.

¹ A total of 1,561 pigs (barrows or gilts) from 140 litters across 5 sow farms were randomly assigned by litter to vaccination treatments at processing (4 d of age). Barrows and gilts were housed in single-sex pens in the nursery with vaccinated and non-vaccinated pigs comingled within each pen. In the finishing barn, pigs were separated by vaccination treatment and gender and a 112-d growth study was performed. There were 24 pens of gilts and 25 pens of barrows. All pens of pigs (1,292 pigs total) were initially weighed 2 wk after placement in the finishing barn (d 0). On d 90, a total of 168 of the heaviest pigs (83 barrows and 85 gilts) were weighed, taken off test, and sold as top pigs. Data from these heaviest pigs were included in pen data and accounted for in calculations. Pen-level performance data were analyzed by analysis of variance using the GLIMMIX procedure of SAS version 9.1.3 (SAS Institute, Inc., Cary, NC). The model included fixed effects of vaccination treatment, gender, and their interaction.

Table 6.3 Means and standard errors for growth performance responses for non-vaccinated control and vaccinated finisher pigs^{1,2}

Item	Control	Vaccinate	Probability, <i>P</i> <
Initial BW on d 0, kg	26.5 ± 0.79	26.6 ± 0.77	0.90
d 0 to 112			
ADG, kg	0.887 ± 0.0121	0.874 ± 0.0119	0.44
ADFI, kg	2.427 ± 0.0368	2.431 ± 0.0361	0.93
G:F	0.366 ± 0.0027	0.361 ± 0.0026	0.13
Mean off-test BW, kg	126.8 ± 1.08	126.8 ± 1.06	0.95

Note. Results are reported as least squares mean ± standard error of the mean.

¹ A total of 1,561 pigs (barrows or gilts) from 140 litters across 5 sow farms were assigned to vaccination treatments at processing (4 d of age) by randomly assigning entire litters to either a vaccinated treatment or a non-vaccinated control treatment. Control and vaccinated pigs were comingled in the nursery and then separated by vaccination treatment and gender in the finishing barn. Treatment pens were randomly distributed throughout the barn. There were 24 pens of control pigs and 25 pens of vaccinated pigs. All pens of pigs (1,292 pigs total) were initially weighed 2 wk after placement in the finishing barn (d 0) and again every 2-wk until d 112. On d 90, a total of 168 of the heaviest pigs (84 control pigs and 84 vaccinated pigs) were weighed, taken off test, and sold as top pigs. Data from these heaviest pigs were included in pen data and accounted for in calculations. Pen-level performance data were analyzed by analysis of variance using the GLIMMIX procedure of SAS version 9.1.3 (SAS Institute, Inc., Cary, NC). The model included fixed effects of vaccination treatment, gender, and their interaction.

² Vaccination treatments were: Non-vaccinated (control) or vaccinated (vaccinate). Vaccinated pigs were injected intramuscularly with a 2-dose (1 mL per dose) porcine reproductive and respiratory syndrome virus subunit vaccine (PRRSV-RS; Sirrah-Bios, Ames, IA) at processing and weaning.